Fifth International Congress of the Federation of the European Societies for Trace Elements and Minerals

TRACE ELEMENTS IN AVIGNON

BRIDGING BETWEEN NEW ADVANCES AND PUBLIC HEALTH ISSUES

Avignon (France) May 22nd - 24th, 2013

FESTEM

SFERETE

FIFTH INTERNATIONNAL CONGRESS OF THE FESTEM

AVIGNON MAY 22ND – 24TH, 2013

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President of the Federation of the European Societies for Trace Elements and Minerals (FESTEM)

Dr Isabelle Hininger–Favier
President of the French-Speaking Society for Studies and Research on Trace Elements and Minerals (SFERETE)

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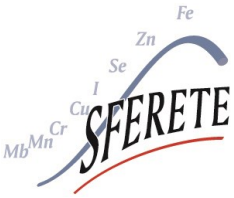

WELCOME MESSAGE FROM THE FESTEM BOARD AND FROM THE FRENCH ORGANIZING COMMITTEE

Dear friends and colleagues

On behalf of the the Federation of the European Societies on Trace Elements and Minerals (FESTEM) Board and the French organizing committee, it is a great pleasure to welcome you in Avignon, France, for the 5th International Symposium of the FESTEM, from May 22nd to 24th, 2013.

Following a tradition initiated almost twelve years ago in Venice in 2001 (Italy), and continued in Munich 2004 (Germany), in Santiago de Compostela 2007 (Spain) and in Saint Petersburg 2010 (Russia), this fifth symposium will bring together a community of researchers in biomedical and environmental disciplines and is aimed at promoting interdisciplinary research discussions, at strengthening collaborations and contacts between scientists of various fields such as biologists, chemists or epidemiologists and clinicians working in the field of trace elements and minerals.

We take this opportunity to thank the previous societies for really stimulating and encouraging trace elements and minerals research in human health, in biology and related fields.

The SFERETE (the French-Speaking Society for Studies and Research on Essential and Toxic Elements) is very honoured to organize the 5th symposium in France. Together with the FESTEM representatives the organizing committee cordially welcomes you in Avignon. Our old and historic host city reflects the facets of middle age and maintains the heritage and traditions of the 'Provence' way of life.

As the symposium title suggests, the Scientific Program of the 5th FESTEM symposium reflects the integrated view that science is facing in this new millennium: The new frontiers of medicine will have to be in close association with other areas of science since many health problems are closely related with occupational and environmental exposure to pollutants. The themes of this meeting

include: Nutritional needs for health, analytical and clinical aspects, and results of environmental and toxicological research.

Hence, the Program of the 5th FESTEM congress includes 15 plenary lectures, 27 short oral communications and a total of 180 papers covering many fields, and addressing clinical, health and environmental issues where trace element play significant roles.

This year the Gabriel Bertrand Award recognizes chromium as the trace element which has focused a great attention and debate during these last 10 years regarding the epidemic-like increase of diabetes cases in the world. We are particularly glad and honoured to reward Dr Richard Anderson who has devoted a large part of his scientific life to chromium research.

The presence of prestigious scientists will be stimulating, specifically for students coming from all over the world, and will be a valuable contribution for the 5th FESTEM symposium to become a high quality symposium.

We are grateful to the members of the Scientific Committee for their expert help in building up the scientific program, to the chairpersons for their participation in the discussions, and to all participants coming from 20 different countries.

We feel indebted to the members of the Local Organizing Committee for their continuous support, their strong commitment and the time they devoted to the congress organisation, and specifically to Mrs Alcaraz, who has been the secretary of the congress.

Lastly, we are grateful to the various organisations, private companies, and to the Grenoble University (Joseph Fourier University) for their financial support to these conferences.

We wish you will attend fruitful conferences and you will have a pleasant stay in Avignon.

Isabelle HININGER-FAVIER
President of Organising Committee
President of the SFERETE

Bernhard MICHALKE
FESTEM President
President of the German society

ABOUT THE FESTEM

The Federation of European Societies on Trace Elements and Minerals (FESTEM) is a nonprofit organization founded in 1997, under the initiative of three societies from Italy (AISETOV), Germany (GSM) and from French speaking countries (SFERETE). Then the SEQC-ET (Spain) and the RUSTEM (Russia) joined the Federation in 2002.

The aim of the Federation is to increase the collaboration, the diffusion and the exchange of information on scientific activities of participating Societies.

The FESTEM aims to encourage all kinds of activities in the field of trace elements and minerals including development of research or educational projects, organization of scientific meetings, search for financial support through private or international organizations, etc.. Detailed information concerning the past and current activities of the FESTEM can be found in the **Journal of Trace Elements in Medicine and Biology**, the official organ of the Federation.

The Federation has been officially registered at the University of Grenoble and at the "*Prefecture de l'Isère*" and the statutes of FESTEM have been published in the "*Journal Officiel*", which is the government journal of publication of the French Republic laws. The executive board consists in two persons per society with a right to vote. Additionally, the editor of the Journal of Trace Elements in Biology is member of the FESTEM board, but he has no right to vote.

The FESTEM is supporting meetings and congresses like the Avignon congress.

THE SFERETE

The SFERETE (Société Francophone d'Etude et de Recherche sur les Eléments Toxiques et Essentiels) is a French non-profit organization working in the field of Trace Elements and Minerals. It has been created by Pr Alain Favier in 1986.

The SFERETE aims at promoting a scientific knowledge in the field of Trace Elements

The actions of the SFERETE concern:

- Information exchanges in the field of Trace Elements
- The publication of books updating the knowledge and gathering research results in the field of Trace Elements
- The organization of national and international meetings and congresses
- Participation in courses for persons working on trace elements
- The attribution of grants and awards
- The initiation of research and formation projects, and particularly in multicentric studies

The Organizers would like to thank the following organisms and firms.

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FIFTH INTERNATIONAL CONGRESS OF THE FESTEM

MAY 22ND – 24TH, 2013

Avignon Grand Hotel
34 boulevard Saint Roch
84000 Avignon
France
+33 (0)490 809 809

Conferences will be delivered in the *Salon du Conclave*

Posters will be displayed in the *Salon Mistral*

SOCIAL PROGRAMME

Tuesday May 21st

Welcome reception from 7:00 pm to 8:00 pm in the Grand Hotel lobby for all participants and accompanying persons

Wednesday May 22nd

7:00 pm – 10:00 pm **Guided Visit of the Popes Palace** followed by a **Cocktail dinner in the *'espace Jeanne Laurent'*** on registration for accompanying persons

Thursday May 23rd

7:30 pm - 10:00 pm **Gala dinner** (only on registration) in the Hotel *Cloitre Saint Louis*

GABRIEL BERTRAND

THE PRIZE

The FESTEM is proud to honour the memory of the great French scientist Gabriel Bertrand - who created the term "trace elements" - with the attribution of the "Gabriel Bertrand Prize" to outstanding scientists in the field of trace element and mineral research. The prize is generously sponsored by the French firm, "Labcatal", which is a leading pharmaceutical company in trace element medicine.

An independent scientific committee nominates the best candidates, and finally chooses one of them.

Laureates of the Gabriel Bertrand Prize

Former winners of the Gabriel Bertrand Prize prize:

- Prof. Dr. T.C. Stadtman, from the National Institutes of Health in Bethesda, received the prize during the 1st FESTEM Symposium 2001 in Venice/Italy
- Prof. Dr. B. Lönnerdal, from the University of California in Davis, received the prize during the 2nd FESTEM Symposium 2004 in Munich/Germany
- Prof. Dr. P. Brätter Em. professor at the Technical University of Berlin and Em. head of the department "Trace Elements in Health and Nutrition" at the Hahn-Meitner Institute, Berlin, received the prize during the 3rd FESTEM Symposium 2007 in Santiago de Compostella/Spain
- Prof. Dr. Vadim Gladyshev from the Department of Medicine, Harvard Medical School and Director of Redox Medicine, Medicine, Brigham and Women's Hospital, received the prize during the 4th FESTEM Symposium 2010 in Saint Petersburg, Russia
- Prof. Dr. R.A. Anderson from the Diet, Genomics and Immunology Laboratory, United States Department of Agriculture (USDA), Agricultural Research Service (ARS), in the USA will receive the prize during the 5th FESTEM Symposium 2013 in Avignon, France

Gabriel Bertrand - A life devoted to trace elements!

Gabriel Bertrand (1867 - 1962) was a French chemist and biologist.

1886-1889	Scholarship in the Edmond Frémy's laboratory
1889-1890	Technician in the Laboratory of Plant Physiology and Applied Agriculture
1890-1897	Chancellor in the Paris museum of Natural History
1894	Award in Experimental Physiology from the Academy of Sciences
1900-1962	Brought Biochemistry in the Pasteur-Institute. Scientific highlights: discovery of oxydases, of the role of manganese in nature, of

	the importance of trace elements, of the nature of enzymes and the mode of enzymatic reactions
1903	Co-founder of the Pasteur-Institute letter
1905-1936	Successor of Emile Duclaux as a Biochemistry professor in the faculty of Natural History in Paris.
1909-1936	Member of various ministerial commissions (hygiene, education, national defense, agriculture and industry).
1914-1918	Support of the industry for national defense
1923	President of the French Chemical Society
1934	Member of the scientific advisory board of the Pasteur Institute. Official retirement: spent all this time in investigations in the Pasteur Institute
1962	Deceased in Paris.

PROGRAMME

TUESDAY MAY 21ST

5:00 pm – 8:00 pm Registration

Welcome ceremony 7:00 pm – 8:00 pm

WEDNESDAY MAY 22ND

8:00 am - 9:00 am Registration

8:45 am - 9:00 am Opening ceremony

Pr B Michalke FESTEM President
Dr I Hininger-Favier SFERETE President

9:00 am - 10:00 am Gabriel Bertrand award (Plenary lecture)
Dr RA Anderson (USA) Chromium in human nutrition

Coffee break & poster session 1 in the '*Salle Mistral*'

10:30 am – 12:30 am Session 1. Trace elements and nutritional needs in Humans
Moderators : J Neve, CW Levenson

Plenary lectures

S Fairweather-Tait (UK)
Requirements for iron, zinc and selenium : the EURRECA experience.

A Shenkin (UK)
Trace Elements in parenteral and enteral nutrition.

Oral communications

AV Skalny - Macro and trace elements for demography and public health improvement: Russian national project

M Vinceti *et al.* - Selenium and human health: bridging together epidemiologic and laboratory evidence

12:30 pm Lunch in the restaurant of the Grand Hotel

2:00 pm – 3:30 pm Session 2. Trace elements, molecular biology and mitochondrial function
Moderators : L Chavatte, E. Mocchegiani.

Plenary lecture

S Sensi (USA)
Physiological and pathological modulation of brain functioning by zinc: role of mitochondria

Oral communications

B Romier *et al.* - Influence of a combination of trace elements on adipocyte biology

W Maret *et al.* - Zinc(II) ions as cellular metallotransmitters in phosphorylation signalling

KTaylor *et al.* – Zn^{+2} signaling in biology and disease.

Coffee break & poster session 1 in the '*Salle Mistral*'

4:00 pm – 5:30 pm Session 3. Advances in trace element assessment.
Moderators : J Arnaud - M Gonzales Estecha

Plenary lectures

E Urrechaga *et al.* (Spain)
Markers of hypochromia in the detection of latent iron deficiency in non-anemic young women.

B Michalke (Germany)
Trace Element Speciation in Brain – Focus Manganese.

Oral communications

N Solovyev *et al.* - Selenium speciation in paired serum and cerebrospinal fluid samples

JI Anetor *et al.* - Strenghtening analytical capability in micronutrients: a prophylactic approach to DNA repair defects, genome instability and carcinogenesis in developing countries.

5:30 pm – 6:30 pm Poster session 1 in the '*Salle Mistral*'

From 7:00 pm to 9:30 pm, participants are invited to a private visit of the 'Palais des papes', followed by a cocktail dinner in the 'Espace Jeanne Laurent'.

THURSDAY MAY 23RD

8:30 am – 10:30 am Session 4. Trace elements in chronic and inherited disorders. Moderators : P Chappuis, S Sensi

Plenary lecture

S Vaulont (France)
New advances in hemochromatosis

Oral communications

J Bertinato *et al.* - Obese rats have higher iron requirements compared to lean rats

S Farida *et al.* – Iron increases the risk of gestational diabetes in pregnant rats

Plenary lecture

JM Trocello et al. (France)
Eurowilson, an European network for Wilson disease.

Oral communications

M Bost *et al.* - Efficiency of molecular analysis of ATP7B gene in diagnosis of Wilson disease

AS Brunet *et al.* - Exchangeable copper : a new promising biomarker for the diagnosis of Wilson's disease (WD)

10:30 am - 11:00 am Coffee break & poster session 2 in the '*Salle Mistral*'

11:00 am – 12:30 pm Session 5. Trace elements in pregnancy and childhood. Moderators : P Van Dael - E Urrechaga

Plenary lecture

CW Levenson (USA)
Role of zinc in development: regulation of stem cell proliferation, survival and differentiation.

Oral communications

J Molnár - Serum selenium concentrations in pregnant women in Hungary: is it time for selenium supplementation?

CM Taylor *et al.* - Effects of maternal lead levels on pregnancy outcomes: the ALSPAC study

K Channa *et al.* - Prenatal exposure to environmental mercury in populations along the coastal region of the Indian ocean in South Africa

12:30 pm Lunch in the restaurant of the Grand Hotel

2:00 pm – 3:00 pm Session 6. Trace elements in ageing
Moderators : A.M. Roussel, R. Anderson

Plenary lectures
J Köhrle (Germany)
Trace Elements and thyroid function in ageing

Oral communications
G Boivin – Effects of strontium ranelate on bones in osteoporotic women

Y Legrain *et al.* Interconnexion between selenium, selenoproteins and cellular senescence

Presentation of the Journal of Trace Element in Medicine and Biology

3:30 pm - 4:00 pm Coffee break & poster session 2 in the '*Salle Mistral'*

4:00 pm – 5:00 pm Session 6. Continued

Plenary lecture
E Mocchegiani (Italy)
Trace element importance for neuroprotection and inflammation

Oral communications
D Oberleas - Diabetes, type II as a nutritional deficiency

I Palazón-Bru *et al.* - Heavy metals and multiple Sclerosis

5:00 pm – 6:00 pm Poster session 2 in the '*Salle Mistral'*

6:00 pm – 7:00 pm Meeting of the FESTEM board

7:30 pm Gala dinner in the Hotel Cloitre Saint Louis *(only on registration)*

FRIDAY MAY 24TH

9:00 am – 11:00 am Session 7. Trace elements in animals
Moderators : D Granjean, M Bost

Plenary lectures

D Grandjean (France)
Trace elements needs and deficiencies in pets.

EA Sizova (Russia)
Biological effects connected with metal nanoparticles into organism

Oral communications

S Metairon *et al.* - Determination of inorganic elements in blood of golden retriever muscular dystrophy dogs using neutron activation analysis

S Amara *et al.* - Effect of TiO_2 nanoparticles on emotional behavior and biochemical parameters in adult Wistar rats

M Anissimova *et al.* - Effect of a newly synthesized manganese salt on the *Eimeria Tenella* (protozoa) experimentally infected broiler chickens

E Humann-Ziehank *et al.* - Long-term study of ovine pulmonary adenocarcinogenesis in sheep with marginal vs. sufficient nutritional selenium supply: results from computed tomography, pathology, immunohistochemistry, JSRV-PCR and lung biochemistry

11:00 am - 11:30 am Coffee break in the '*Salle Mistral*'

11:30 am – 12:30 pm Session 8. Trace elements in Dermatology
Moderators : A Skalny, I Hininger-Favier

Plenary lecture

MT Leccia (France)
Trace elements in photoprotection.

Oral communications

W Rachidi *et al.* - Evaluation of age impact on the protective effect of selenium against UVA irradiation in primary human keratinocytes

MG Skalnaya - Copper deficiency - a new reason of androgenetic alopecia?

12:30 pm Lunch in the restaurant of the Grand Hotel

2:00 pm – 3:30 pm Session 9. Trace elements in environment.
Moderators : P Borrela, B Michalke

Plenary lecture

JM Ibarluzea Maurolagoitia (Spain)
Heavy metals in public health issues: environment and childhood (infencia y medio ambiente) project: neurodevelopment questions and mercury

Oral communications

S Barhoumi *et al.* - Use of oxidative stress biomarkers in *Salaria Basilisca* for assessing heavy metals pollution in the gulf of Gabes

B Kocer *et al.* – The protective effect of organic and inorganic selenium against the oxidant potential of monocyclic aromatic amines in different cellular fractions

M González-Estecha *et al.* – Study of serum cerium and its relationship with atmospheric pollution caused by suspended particulate matter (PM 2.5) in Madrid.

Closing ceremony & poster awards

Pr B Michalke FESTEM President
Dr I Hininger-Favier SFERETE President

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Website: www.merck-medication-familiale.fr

Abstracts of the plenary lectures and oral communications

PL-1

CHROMIUM IN HUMAN NUTRITION

Richard A. Anderson

Diet, Genomics and Immunology Laboratory
Beltsville Human Nutrition Research Center
Beltsville, Maryland, USA 20705

Documentation of the essential role of trivalent Cr in human nutrition was first demonstrated when a 40 y old female, who had been on total parenteral nutrition for 5 years, developed severe signs of diabetes including weight loss, glucose intolerance, and insulin resistance. The addition of 250 µg of Cr to her TPN solution led to the reversal of all these signs and symptoms of diabetes within two weeks. Signs of Cr deficiency in the general population are much less severe and are often similar to those of the metabolic syndrome. Several human studies have demonstrated the beneficial role of Cr in normal free-living subjects. A human study involving 155 people with type 2 diabetes demonstrated beneficial effects of Cr on fasting glucose, glucose tolerance, insulin, cholesterol and HbA1c in a dose response manner. These results have been substantiated in several laboratories. Cr has also been shown to affect weight loss and body composition but these studies remain controversial. Stresses including high sugar intake, acute exercise, burn injury, physical trauma and steroid administration have all been shown to increase Cr losses. Severe insulin resistance associated with steroid administration has been shown to be reversed by supplemental Cr. Cr has also been shown to improve cognition in people with mild cognitive impairment and changes in the brain due to Cr have been demonstrated using fMRI. However, not all studies involving Cr are positive and response to Cr varies based upon the selection of subjects, degree of glucose intolerance, form of Cr tested, duration of the study etc.. Cr is a very safe nutrient and essentially no toxic effects have been documented in studies involving supplemental Cr. In summary, signs of Cr deficiency are similar to those of the metabolic syndrome and numerous human studies have reported beneficial effects of supplemental Cr. It is often not clear if the beneficial effects of Cr are nutritional or therapeutic effects.

PL-2

REQUIREMENTS FOR IRON, ZINC AND SELENIUM: THE EURRECA EXPERIENCE

Susan J Fairweather-Tait

Department of Nutrition, Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, UK
s.fairweather-tait@uea.ac.uk

Partners in the EC-funded Network of Excellence, EURopean RECommendations Aligned (EURRECA), have recently undertaken a programme of work on dietary requirements for micronutrients, with a particular focus on the derivation of dietary reference values. Initially, suitable methodologies were identified for measuring dietary intake, status (biomarkers of exposure and nutritional status), and health endpoints / outcomes. A method was devised to select priority micronutrients which included consideration of public health concerns in Europe, publication of new information (subsequent to the 1993 Report of the Scientific Committee for Food on nutrient intakes for the EC), and disparity in values between different recommendation setting bodies. A series of systematic reviews were carried out using harmonised approaches and selecting the highest quality data, according to the established best practice guidelines, to examine the relationships between measurements of intake, status and health for selected micronutrients, and to collect data required for the factorial approach for estimating requirements (losses, growth, bioavailability etc). The overall aim of the work was to collate and combine all published data of sufficient quality in order to carry out meta-analyses and devise dose-response relationships. The main conclusions relating to iron, zinc and selenium requirements in adults will be presented. This work has been carried out with partial financial support from the Commission of the European Communities, specific RTD Programme "Quality of Life and Management of Living Resources", within the 6th Framework Programme (Contract No. FP6-036196-2 EURRECA: EURopean micronutrient RECommendations Aligned) and does not necessarily reflect the views of the Commission and in no way anticipates the future policy in this area.

PL-3

TRACE ELEMENTS IN PARENTERAL AND ENTERAL NUTRITION

Alan Shenkin

Emeritus Professor of Clinical Chemistry, University of Liverpool, England shenkin@liv.ac.uk

Effective artificial nutrition by parenteral or enteral route requires the provision of a safe and adequate amount of all essential trace elements. Enteral nutrition products meet or exceed Reference Nutrient Intakes but many questions still remain in parenteral nutrition (PN). This presentation will focus on those issues in adults.

There are good recommendations for zinc and copper in stable patients (3-4mg/day, and 0.3-0.5mg/day respectively), and additional amounts for those with increased losses. The elements about which there is most debate are selenium, manganese, chromium and iron. Until recently, the Se recommendation was 20-60µg/day, but a basal amount of 60µg/day is now suggested, increasing to 100µg/day in patients who are depleted or with increased oxidative stress. Provision of high dose Se to patients in intensive care is controversial with 500-1000µg/day being suggested by some workers. Provision of Mn has fallen over the past 20years from 2mg, to a recommendation of 55µg/day as a result of cases of Mn toxicity, and lack of convincing evidence of cases of Mn deficiency.

There is uncertainty regarding the requirement for Cr during PN. All patients receiving PN have markedly elevated serum and tissue Cr concentration as a result of Cr contaminants in the regimen. Cr provision to neonates is not recommended due to possible renal toxicity, but this has not been observed in adults. Whether Cr should be provided, and the optimal dose in adults is therefore not clear. The daily parenteral requirement for iron is about 1mg and this is provided in most European additives, but not in those in USA due to concern about stability of fat emulsions. The other essential elements, molybdenum, iodine and fluorine, are also usually included in Europe, but not in the USA due to lack of evidence of deficiency states during PN.

Future work will focus mainly on the optimal provision of Se, Cr and Mn and on the bioavailability of contaminants in the PN regimen.

OC-1

MACRO AND TRACE ELEMENTS FOR DEMOGRAPHY AND PUBLIC HEALTH IMPROVEMENT: RUSSIAN NATIONAL PROJECT

A.V. Skalny [1,2]

[1] *Trace Element – Institute for UNESCO, Lyon, France*
[2] *Russian Society of Trace Elements in Medicine, Moscow, Russia*
E-mail: skalny3@microelements.ru

In frame of a national project, induced by TEU satellite center in Orenburg State University, Institute of toxicology (St.Petersburg) and RUSTEM, near 100000 people, living in different regions of the European part of Russia, were investigated. Scalp hair (SH), whole blood (WB), serum (BS) and morning urine (MU) samples were collected and analyzed by ICP-MS. There were significant differences between the almost all regions in SH multielement profiles, but the WB, BS and MU profiles were very similar. Comparison of SH, WB, BS and MU profiles of Russian population to published reference ranges of EU population showed the lower levels of some essential elements (Zn, Cu, Co) and relatively higher – of some toxic metals (Ni, Mn, Cd), especially in children. The maximal rate of deviations in elemental status were observed in northwest part of Russia (republics of Komi, Karelia, Arkhangelsk region) and minimal ones - in central part (Orel, Ryazan, Kaluga, Moscow regions) and south part (Krasnodar, Stavropol, Dagestan). Obtained SH data were well corresponding to environmental pollution of regions, mineral content of drinking water and climato-geografical peculiarities, but not to life standards. Low hair Ca, P, K, Na, Co, Se levels and elevated Fe, Mn, Cr, Hg correlated directly to decrease of life expectance. Men were more susceptible to K, Na, Ca, P, Se deficiencies or imbalances, but women - to lack of Co and Mn excess. Elevated hair Zn and K in male population significantly correlates with increased birth rate. Mortality rate directly correlates to low hair Ca, Mg, K, P and excess Hg, Mn in both sexes. The total morbidity of population closely corresponds to the combination of Ca, P, Mg deficiencies and Hg, Pb, As, Al, Cd excess. So, an increase in macro and some trace elements provision and diminishing of toxic metals, Fe, Mn accumulation in population can improve demographic data in regions and in whole Russian Federation.

OC-2

SELENIUM AND HUMAN HEALTH: BRIDGING TOGETHER EPIDEMIOLOGIC AND LABORATORY EVIDENCE

Marco Vinceti, Annalisa Bargellini, Carlotta Malagoli, Paola Borella

Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia, Modena, Italy

We briefly review the current evidences concerning a key public health issue, the beneficial and toxic health effects of selenium exposure, by critically analyzing results of the most recent epidemiologic and laboratory studies on this topic. The relation of this metalloid to human health, cancer in particular, is puzzling: a recent large trial SELECT and a meta-analysis by the Cochrane Collaboration found no clear evidence that selenium supplementation in Western populations has beneficial effects on cancer prevention, contrary to what suggested by earliest experimental and observational cohort studies. In addition, it has not been established yet if very low or high selenium dietary intakes are associated to increased cancer risk, and in such case at which amounts of exposure such effects may occur. Low and high levels of selenium exposure have also been linked to risk of Keshan disease, a rare cardiomyopathy described in some Chinese areas, and to type-2 diabetes and amyotrophic lateral sclerosis, respectively. Investigations under both an epidemiologic and a biochemical perspective are currently ongoing on these issues, in order to better identify the diseases actually associated to an abnormal selenium status, assess the safe range of intake of this metalloid, and establish more appropriate public health standards taking into account both the toxicological and the nutritional aspects. Key issues of such research activity are the identification of more adequate indicators of selenium exposure, and the awareness that the various inorganic/organic selenium compounds may have different biological activities, thus emphasizing the relevance of selenium speciation in investigating the effects of this metalloid on human health.

PL-4

PHYSIOLOGICAL AND PATHOLOGICAL MODULATION OF BRAIN FUNCTIONING BY ZINC: ROLE OF MITOCHONDRIA

S Sensi (USA)

Abstract not received

OC-3

INFLUENCE OF A COMBINATION OF TRACE ELEMENTS ON ADIPOCYTE BIOLOGY

Béatrice Romier[a,b,c‡], Franck Tourniaire [a,b,c‡], Julie Marcotorchino [a,b,c], Erwan Gouranton [a,b,c], Julien Astier [a,b,c], Christiane Malezet [a,b,c], Eric Blouin[d] and Jean-François Landrier [a,b,c] *

[a] *INRA, UMR_INRA 1260, Marseille, 13005, France*
[b] *INSERM, UMR_S 1062 « Nutrition Obésité et Risque Thrombotique », Marseille, 13005, France;*
[c] *Aix Marseille Université Marseille, 13005, France*
Fax: +33 4 91 78 21 01; Tel:+33 4 91 29 41 17 jean-francois.landrier@univ-amu.fr
[d] *Laboratoires Labcatal, 7 rue Roger Salengro 92541 Montrouge, France. Fax: +33 1 46 54 22 21; Tel: +33 1 46 54 27 92.*
[‡] *These two authors contributed equally to this work.*

The white adipose tissue is playing a major role in the development of obesity and associated metabolic complications by producing a variety of pro and anti-inflammatory adipokines and adipocytokines. Recently, studies in humans or in animals have shown a beneficial effect of certain trace elements such as zinc on insulin resistance and adipokine secretion. The aim of our study was to test the effect of a Zinc-Nickel-Cobalt solution (ZnNiCo) on adipocyte function and to identify potential health effects of this solution in the context of obesity and associated disorders.

The effect of ZnNiCo on adipocyte differentiation process was investigated. Gene expression in murine and human TNF-α stimulated adipocytes was examined in the presence of ZnNiCo using whole genome microarrays. Data were validated in animals fed a high fat diet supplememented with ZnNiCo. Finally, the impact of ZnNiCo on activation of NF-κB was evaluated by ELISA.

No impact of ZnNiCo on adipogenesis was observed in 3T3-L1 cells. Transcriptomic analysis indicated that ZnNiCo affected the expression levels of genes in adipocytes in basal condition or incubated with TNF-α and shown a down regulation of several inflammatory genes belonging to the cytokine and chemokine families. These data were confirmed in mice. A modulation of NF-κB activation by ZnNiCo could explain at least in part these observations.

Trace elements that are contained in ZnNiCo can modulate the expression of several inflammation related adipocytes transcripts. This study suggests that ZnNiCo solutions might prevent obese adipose tissue from inflammation.

OC-4

ZINC(II) IONS AS CELLULAR METALLOTRANSMITTERS IN PHOSPHORYLATION SIGNALLING

Wolfgang Maret* and Christer Hogstrand

King's College London, Diabetes and Nutritional Sciences, Metal Metabolism Group, 150 Stamford Street, London, SE1 9NH, United Kingdom; wolfgang.maret@kcl.ac.uk

With functions in about 3000 human proteins, the rôle of zinc in biology is already preeminent among the micronutrients. Yet, biological zinc research has entered another stage of discovery with the realization that zinc(II) ions are intercellular and intracellular messengers. Zinc(II) ions are stored in subcellular compartments and are released through specific signalling pathways. The resulting cellular zinc(II) ion transients occur at remarkably low concentrations (picomolar) and target protein tyrosine phosphatases, such as PTP-1B and RPTPbeta, enzymes involved in diabetes, cancer, angiogenesis and wound healing. Based on measured cytosolic free zinc(II) ion concentrations, the very strong zinc inhibition of RPTPbeta (K_i = 21 pM) indicates tonic inhibition and a requirement for activation by zinc chelation. The data suggest that zinc regulates cellular phosphorylation cascades by targeting enzymes that are not recognized as zinc enzymes because they are commonly isolated in the presence of chelating agents to preserve their enzymatic activities. These latest developments have significant implications for cellular control and communication, ageing, and the pathophysiology of degenerative diseases. Overall, the findings link trace element research and mainstream cellular biology and provide ample new opportunities to determine how zinc deficiency affects the balance between health and disease.

OC-5

ZN^{2+} SIGNALLING IN BIOLOGY AND DISEASE

Kathryn Taylor[1], Wolfgang Maret[2] and Christer Hogstrand[2,*]

[1]*Breast Cancer Molecular Pharmacology Group, School of Pharmacy and Pharmaceutical Sciences, Cardiff University, King Edward VIIth Avenue, Cardiff, CF10 3NB, Wales, UK*
[2]*King's College London, Diabetes and Nutritional Sciences, Metal Metabolism Group, 150 Stamford Street, London, SE1 9NH, United Kingdom; christer.hogstrand@kcl.ac.uk*

Zinc is used as a structural or catalytic component in about 3000 proteins, representing about 10% of the genome. However, the zinc(II) ion is also a signalling molecule with regulatory roles in numerous cellular and systemic processes. Zinc channels and transporters are key components on zinc signalling processes as they control intracellular concentrations of zinc at the level of organelle compartments. Two closely related zinc channels, ZIP6 and ZIP10 (SLC39A6, SLC39A10), are located at the plasma membrane and mediate movement of extracellular zinc(II) into the cytosol. Zinc(II) influx through these channels is regulating the epithelial-mesenchyme transition (EMT) during embryo development and stimulates cell migration. These proteins are highly expressed in breast cancer cells, where they also stimulate EMT through activation of AKT-GSK3ß signalling, hence, contributing to cancer invasiveness. Another protein from the same family, ZIP7 (SLC39A7), mediates gated zinc(II) flux from the endoplasmic reticulum to the cytosol in response to serine-phosphorylation by CK2. The resulting zinc(II) wave triggers downstream signalling events through increased phosphorylation of kinases, such as AKT and ERK1/2. Because, this gated zinc(II) release can be triggered by extracellular stimuli, the zinc(II) ion fulfils all criteria of a second messenger.

PL-5

MARKERS OF HYPOCHROMIA IN THE DETECTION OF LATENT IRON DEFICIENCY IN NON-ANEMIC YOUNG WOMEN

E Urrechaga [1], L Borque [2], J F Escanero [2]

1 Laboratory. Hospital Galdakao-Usansolo Galdakao, Vizcaya SPAIN
2 Department of Pharmacology and Physiology. Faculty of Medicine. University of Zaragoza, Zaragoza SPAIN
e-mail: eloisa.urrechagaigartua@osakidetza.net

Background: Iron deficiency is the most common cause of anemia in fertile women but a hemoglobin (Hb) level within the reference interval does not exclude Iron deficiency. Deficiency occurs in progressive stages, initially with a negative balance between iron stores and the requirement for erythropoiesis, leading to a progressive depletion of stores and eventually to anemia.

The aim was to assess the reliability of the extended hemogram by the Sysmex XE 5000 reporting reticulocyte Hb equivalent (RetHe) and percentage of hypochromic erythrocytes (%HypoHe), in the detection of latent iron deficiency (LID), defined as iron depletion without anemia.

Methods: Two hundred and fifty non- anemic women in fertile age (18-40 years), whose analyses had been requested by general practitioners, were included in the study. One hundred and fifty three had ferritin within reference range and Hb> 120 g/L; 97 had LID (depletion of iron reserves without anemia), defined by Serum ferritin < 20 µg/L and Hb > 120 g/L.

Samples were analyzed for full blood count, serum ferritin and serum iron. The diagnostic performance in the discrimination between healthy females and those with LID was assessed with Receiver operating characteristic (ROC) curve analysis and concordance with Cohen's Kappa Index.

Results: ferritin AUC 0.981, cut off 24 µg/L Sensitivity 90 % Specificity 95.8 %; %HypoHe AUC 0.924, cut off 0.9 % Sensitivity 85 % Specificity 92.5 %; ferritin AUC /%HypoHe AUC P=0.854; RetHe AUC 0.902, cut off 31.0 pg, Sensitivity 75 % Specificity 94.7 %, ferritin AUC /RetHe AUC P=0.085. Applying those cut offs the agreement between ferritin and %HypoHe was κ 0.61 and 0.56 for ferrtin and RetHe.

Conclusions: The diagnosis of mild forms of ID and the early stage of depletion of iron stores presents a great challenge. %HypoHe and RetHe emerge as reliable tests for the investigation of LID and could improve the ability to detect iron deficiency before anemia is present.

PL-6

TRACE ELEMENT SPECIATION IN BRAIN – FOCUS MANGANESE

B. Michalke

Helmholtz Zentrum München – German Research Center for Environmental Health
Research Unit: Analytical BioGeoChemistry
E-mail: bernhard.michalke@helmholtz-muenchen.de

Neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD) are gaining increasing relevance in our aging society. However, their complex multi-factorial mechanisms are not clearly understood. Metal ions can cause neurologic dysfunction: Al could facilitate AD, Pb and Hg are proven to affect mental health. Mn is of special concern in environmental- and occupational medicine: Chronic or excessive Mn exposure is a cause for neurological dysfunction like limitation of mental ability, reduction of psychomotor coordination, damage of the extrapyramidal nervous system and Mn dependant PD ("Manganism"). Often not the metal ions *per se* are the detrimental agent, but specific forms (metal species). As an example absorbed Mn is metabolized in the liver. The newly formed Mn species are transported by blood to brain. Under physiological condition, transferrin (TF) is the main plasma Mn-carrier and the TF-receptor (TF-R) mediated Mn transport across the neural barrier (NB) is limited and strictly controlled. Under excess Mn, different Mn species are generated crossing the neural barriers independently on TF-R. These Mn compounds appear rapidly in cerebrospinal fluid (CSF). On the cellular level, the neurotoxic mechanism of Mn is still not completely clear. Common explanations comprise reactive oxygen species generation, caused by various oxidation states of Mn or by disturbance of cellular Fe and Ca homeostasis. This contribution starts with an overview on various metals speciated in brain or CSF. It then focuses on Mn speciation in Relationships between serum- and CSF-Mn-species are draw. Confirmation of the results is shown with Mn speciation in serum and brain of rats after defined Mn exposure. Possible mechanisms of neural damage are discussed with respect to speciation of further metals at neural barriers and their possible imbalances.

OC-6

SELENIUM SPECIATION IN PAIRED SERUM AND CEREBROSPINAL FLUID SAMPLES

Nikolay Solovyev[1], Achim Berthele[2] and Bernhard Michalke[3]

1 *Institute of Toxicology, FMBA, 192019 St. Petersburg, Russia*
e-mail: nicksolovev@gmail.com
2 *Department of Neurology, Klinikum rechts der Isar, Technische Universität München*
e-mail: berthele@lrz.tu-muenchen.de
3 *Helmholtz Center Munich – German Research Center for Environmental Health*
e-mail: bernhard.michalke@helmholtz-muenchen.de

Se-speciation was performed in 24 individual paired serum and cerebrospinal fluid (CSF) samples from neurologically healthy persons. Strong anion exchange (SAX) separation, coupled to inductively coupled plasma – dynamic reaction cell- mass spectrometry was employed. Species identification was done by standard matched retention time, standard addition and 2-D separation techniques. Limit of detection (LoD, 3 x standard deviation of noise) was in the range of 0.026 - 0.031 µg/L for all investigated species. Quality control for total Se determination was performed by analyzing control materials "human serum" and "urine", where determined values met target values.

Several Se species were found in both sample types having following median values (sequence: serum / CSF, each in µg Se/L): total Se: 58.39 / 0.86; selenoprotein P (SePP): 5.19, 0.47; Se-methionine (SeM): 0.23 / < LoD; glutathione peroxidase (GPx): 4.2 /, 0.036; thioredoxinreductase (TrxR) 1.64 / 0.035; Se(IV) 12.25 / 0.046; Se-human serum albumin (Se-HSA) 18.03 / 0.068. For other Se-species (Se-cystine, Se (VI) and up to four non-identified compounds) median values were <LoD.

Linear relationships based on median values provide information about Se-species passage across neural barriers (NB).In serum a leading influence on total Se-serum was seen only for SePP-serum when total Se was > 65 µg/L. The previously described independence of total Se-CSF on total Se-serum and of $SePP_{\text{-CSF}}$ on $SePP_{\text{-serum}}$ was confirmed.

Linear relationships of Se-compounds between serum and CSF were found only for GPx, Se-HSA and TrxR. Although, $HSA_{\text{-CSF}}/HSA_{\text{-serum}}$ values from the samples were in the normal range for adults, the increased $TrxR_{\text{-CSF}}/TrxR_{\text{-serum}}$ and $GPx_{\text{-CSF}}/GPx_{\text{-serum}}$ ratios are not completely explained by molecular size dependence. Thus, there may be either a facilitated diffusion across NB for anti-oxidative Se-enzymes or they might be additionally expressed in brain.

OC-7

STRENGHTENING ANALYTICAL CAPABILITY IN MICRONUTRIENTS: A PROPHYLACTIC APPROACH TO DNA REPAIR DEFECTS, GENOME INSTABILITY AND CARCINOGENESIS IN DEVELOPING COUNTRIES.

Anetor JI, Iyanda, AA, Akinseye I, Anetor GO*

Toxicology & Micronutrient Metabolism Unit
Department of Chemical Pathology, College of Medicine &
**Department of Human Kinetics & Health Education, Faculty of Education*
University of Ibadan, Nigeria.

Correspondence: anetorji@yahoo.com

DNA repair defects and genome instability may be occult in developing countries. DNA damage is enhanced during increased exposure to chemicals, a common occurrence in industrializing developing countries. Unrepaired damage leads to inactivation of tumor suppressors or activation of oncogenes and mutation that may replicate, leading to genome instability and ultimately carcinogenesis. Certain mechanisms are prophylactic against this. The enzyme 8-oxoguanine DNA glycoxylase (OGG1) repairs DNA bases. Determination of OGG1 demonstrated that individuals with lung cancer carried low levels of this enzyme compared with healthy population. Individuals exposed to environmental pollutants having low OGG1 activity are at increased risk compared with subjects with normal OGG1 level. OGG1 is a member of inadequately known number of DNA repair systems. Low level of any of these repair systems could increase risk of developing cancer. Reports also suggest that DNA damage induced by micronutrient deficiency accounts for over 30% of preventable cancers. Micronutrients act directly or indirectly on the genome to prevent mutations by acting as enzyme cofactors in cellular processes that modulate cellular transformation, DNA damage and repair pathways. Nutrient associated DNA damage response pathways could be pragmatic intervention strategies against genome instability that may progress to cancer considered to be a greater burden in developing countries currently. Limited analytical capability to accurately determine biomarkers of DNA repair pathway defects, genome instability as well as markers of oxidative DNA damage dependent on micronutrients remains a major constraint in these countries. Strenthening them in this regard may prove to be a veritable prophylactic approach to occult DNA repair defects, genome instability and ultimately cancer.

PL-7

NEW ADVANCES IN HEMOCHROMATOSIS

Sophie Vaulont

INSERM, U1016, Institut Cochin, Faculté de Médecine Cochin Port Royal – F-75014 Paris
sophie.vaulont@inserm.fr

Maintaining normal iron homeostasis is essential for the organism as both iron deficiency and iron excess are associated with cellular dysfunction.

In mammals, iron homeostasis is complex and depends on the regulated absorption of dietary iron by mature enterocytes of the duodenum and iron recycling by macrophages, which supply most of the serum iron through recovery of the metal from senescent erythrocytes. These two fundamental processes are regulated by the iron-regulatory hormone hepcidin. This hormone, produced mainly by the hepatocytes, regulates cellular iron efflux by binding to the exporter ferroportin, inducing its internalization and subsequent degradation. Hepcidin is produced under the control of different signals that either induce (increased plasma and tissue iron and inflammation) or repress (erythroid activity, hypoxia, iron deficiency) its synthesis. Recent evidence suggest that low hepcidin levels are responsible for the most frequent inherited disorders world-wide, β-Thalassemia and hereditary hemochromatosis (HH). HH is a heterogeneous genetic disease characterized by excessive iron accumulation in the liver and parenchyma. Clinical manifestations include liver cirrhosis, diabetes, cardiomyopathy, arthropathy, and hepatocellular carcinoma. HH is typically caused by mutations in genes encoding either upstream signaling molecules involved in the induction of hepcidin expression (HFE; transferrin receptor 2; hemojuvelin), or more rarely in the hepcidin gene itself or its receptor, ferroportin. Recent evidence suggest that, in addition to the systemic hepcidin-mediated control, local enterocytes regulators such as oxygen tension and intracellular iron levels, could play important role in regulating iron absorption.

This presentation will outline recent advances in iron regulatory pathways and the important implication of the hepcidin-ferroportin axis in therapeutics.

OC-8

OBESE RATS HAVE HIGHER IRON REQUIREMENTS COMPARED TO LEAN RATS

Jesse Bertinato (Gesse.bertinato@hc-sc.gc.ca), Cristina Aroche (caroco74@uottawa.ca), Louise J. Plouffe (louise.j.plouffe@hc-sc.gc.ca), Megan Lee (meglee88@gmail.com), Zehra Murtaza (zehra.mo26@hotmail.com), Laura Kenney (laura.kenney@hc-sc.gc.ca), and Alfred Aziz (alfred.aziz@hc-sc.gc.ca).

Bureau of Nutritional Sciences, Health Canada, Ottawa, Canada.

Obesity has reached epidemic proportions in Canada and is a major cause of preventable morbidity and mortality. Obesity has also been associated with poorer iron status. The objective of this study was to determine the effects of diet-induced obesity on iron requirements and metabolism. Six-week-old male obese-prone (n=12/diet group) and obese-resistant (n=12/diet group) rats were fed 1 of 4 high-fat diets supplemented with 5 (low), 15 (marginal), 35 (normal) or 70 (high) mg iron/kg diet for 12 weeks. Prior to necropsy, body composition was measured and rats in each diet group were categorized as lean (8.5%-16.9%) or obese (19.4%-35.9%) based on percent body fat. Iron status was assessed by haematological parameters and serum and tissue iron concentrations. Expression of the iron regulatory hormone hepcidin and the intestinal iron transporters Divalent Metal Transporter 1 (DMT-1) and Ferroportin 1 (FPN-1) were determined by Western blotting and/or QPCR. Obese rats fed the low or marginal iron diets showed poorer iron status compared to lean rats despite larger food intakes. Obese rats fed the low iron diet had lower ($P<0.05$) haemoglobin and serum iron, while those fed the marginal iron diet had lower ($P<0.05$) liver iron. Liver hepcidin mRNA content and duodenal expression of DMT-1 and FPN-1 mRNA and protein did not differ between obese and lean rats within each diet group. Iron in duodenal mucosal scrapings and the spleen also did not differ. These data show that obesity increases iron requirements in rats. The results suggest that the higher iron requirements were not a result of reduced intestinal iron uptake or altered iron metabolism in the spleen.

OC-9

IRON INCREASES THE RISK OF GESTATIONAL DIABETES IN PREGNANT RATS

Sitti Farida, 2 , Karine Couturier,1, 2 , Mireille Osman,
Cécile Batandier,1,2, Isabelle Hininger-Favier,1,2

[1] *Université Joseph Fourier, Laboratoire de Bioénergétique Fondamentale et Appliquée, Grenoble, France*
[3] *Inserm U1055, Grenoble, France - farida_q12@yahoo.co.id*

The incidence of gestational diabetes is increasing and might exceed 16% according to the new recommendations for screening. Recently, observational studies reviewed the link between iron, nutritional status and gestational diabetes mellitus (GDM). Despite pregnant women are often iron supplemented, the mechanism of this effect is still not fully documented. Iron overload could be implicated as a triggering mechanism by producing reactive oxygen species. We performed an experimental study in pregnant rats fed three different diets: a control (C), a fructose enriched diet (F) and a fructose + iron enriched diet (F+I). The (F) diet inducing an insulin resistance. Anthropometric, biochemical and oxidative stress parameters were evaluated at delivery and in pup livers. The number and the gender of pups were not different within the different groups. Glycaemia was significantly higher in F and F+I groups than in control group. The highest values were observed in F+I (9,06 ± 0,61 vs 8,96 ±0,98 vs 7.66 ±0,46 mmol/l for F+I, F and C respectively). The same effect was noticed for plasma triglycerides. We found a non significant trend in plasma GPx which increased, suggesting an adaptative mechanism. The thiol groups and the total antioxidant capacity were not modified. Interestingly, glycemia of pups from the (F) and (F+I) groups were significantly lower as compared to (C). Body weights were also significantly higher. Furthermore liver weights were lower in the (F) and (F+I) groups than in (C), with the lowest levels in the (F). Plasma glutathione concentrations were also significantly lower in the F and F+I groups than in (C). This effect being enhanced in male as compared to female pups. These preliminary results suggest that a high iron status increased the risk of GDM and could have a negative impact on pups. These results might contribute to a debate about the possible deleterious effects of iron supplementation in case of risk of gestational diabetes.

PL-8

EUROWILSON: A EUROPEAN NETWORK FOR WILSON DISEASE

Trocello J-M (a,b), Emeline Ruano (a), France Woimant (b)

a EuroWilson. Jean-Marc.trocello@lrb.aphp.fr
b Centre national de référence pour la maladie de Wilson, France

EuroWilson (EW) is a multi-stakeholder network with 67 scientific country coordinators from 19 countries. Its mission is to improve the quality of care and access to multidisciplinary expertise for European patients with Wilson's disease (WD). The main objective is to shorten the delay to diagnosis and to decrease the inequalities between each European country. EW has initiated lots of projects to reach these goals. To facilitate the access for the physicians to more detailed information about WD, we developed a EW website with information about the disease and European experts. We shared experiences during meeting and phone conferences between health professionals and we published on line a quarterly Wilson Journal Watch. EW Network participated to European guidelines.

We realized a registry with newly diagnosed patients since 2005 to increase the knowledge about the disease and to evaluate the feasibility of a therapeutic trial in this rare disease.

Patient's empowerment is also very important in the organisation of this European network. We realized with the help of patient representatives a survey to better define patients priorities and expectations from a European network. This survey has been translated into 5 languages which allowed patients from France, Germany, Italy, England and Spain to fill in the form. Physicians from EW and patient organisations were in charge of the dissemination of this survey. The results of this survey underline a real inequality for the management of this disease and the necessity to improve the collaboration between local and national organisations. The European network takes into account of this conclusion to better answer to patient's expectations.

Registry, e-health and patient's empowerment are some example of EW actions. This European network needs sustainability to achieve their objectives for this treatable disease, if the diagnosis is realized early.

OC-10

EFFICIENCY OF MOLECULAR ANALYSIS OF ATP7B GENE IN DIAGNOSIS OF WILSON DISEASE

Bost M[1,2], Collet C[3], Brunet AS[2], Lachaux A[2], Morineau G[3], Piguet-Lacroix G[1], Trocello JM[2], Woimant F[2], Chappuis Ph [2,3]

1 *Laboratory of Hereditary Diseases of Metabolism, CBPE, Lyon - France muriel.bost@chu-lyon.fr*
2 *Reference Centre for Wilson Disease - France*
3 *Biochemistry and Molecular Biology, Lariboisiere hospital, Paris – France philippe.chappuis@lrb.aphp.fr*

Background: Wilson disease (OMIM#277900) is due to mutations in ATP7B gene (MIM#606882; NG_008806.1; NM_000053.3). Molecular analysis therefore provides the potential for more reliable diagnosis, particularly since an effective treatment is available.

Objectives: To evaluate the efficiency of molecular genetic analysis in diagnosis of patients affected of WD.

Methods: We examined 227 unrelated WD patients whose diagnosis was based on different criteria as the presence of hepatic or neurological symptoms, a Kayser-Fleisher ring, raised urinary copper (Cu) excretion, decreased serum ceruloplasmin and serum Cu levels or sometimes raised hepatic Cu content. Patients registered at the French Reference Centre for Wilson Disease were requested to sign an informed consent form. After salt extracting DNA, direct sequencing of the 21 exons of ATP7B gene was performed and MLPA analysis for large gene rearrangement study made in patients presenting only one or no ATP7B mutation.

Results: Among a cohort of 225 WD unrelated patients, two mutations were identified in ATP7B in 200 WD cases (88,9%), only one in 14 cases (6,2%) and no mutation at all in 11 cases (4,9%). Fourteen WD cases have been screened with MLPA technique. In 5 WD patients, we identified the deletion of an exon. We described new variants in ATP7B gene.

Conclusions: Direct sequencing for ATP7B mutation analysis and MLPA assay lead to the detection of about 92% of the mutated chromosomes. It is probable that, besides exceptional exon copy number or technical problems arising from sequencing methods, other point-mutation located in introns quite far from intron-exon junctions and 3'UTR region could account for the 5-10% non evidenced mutations. This molecular study of WD identified the spectrum of disease-causing mutations in the French patients affected of WD. Documentation and characterization of all variants is essential for accurate DNA diagnosis in WD because of the wide range of clinical and biochemical variability.

OC-11

EXCHANGEABLE COPPER: A NEW PROMISING BIOMARKER FOR THE DIAGNOSIS OF WILSON'S DISEASE (WD)

Brunet AS[1], Guillaud O[1], Mallet I[2], Lachaux A[1], Bost M[1,2]

1 *Reference Centre for Wilson Disease, HFME, Lyon – France, anne-sophie.brunet@chu-lyon.fr*
2 *Trace Element Analysis Laboratory, Biochemistry and Molecular Biology, Edouard Herriot Hospital, Lyon, France, muriel.bost@chu-lyon.fr*

Background : WD diagnosis can be challenging and is based on several clinical and biological features. Measuring of the serum exchangeable copper (CuEXC) seems to be a promising tool. A recent preliminary study showed that relative exchangeable copper (REC), defined by ratio CuEXC / Total serum copper (CuT), > 18.5% had a sensibility (Se) and a specificity (Sp) of 100% for the diagnosis of WD. To date, there is no data regarding exchangeable copper levels in cohorts of patients suffering from hepatic (non wilson) diseases.

Aim : The aim of our study is to determine the sensitivity and specificity of REC for the diagnosis of WD among a group of patients suffering of WD at diagnosis or who fail to respond to treatment (group 1, n=6), a group of Wilsonian patients on stable condition with medical treatment (group 2, n=31), and 2 groups of patients followed for non wilsonian hepatic diseases in an adult hepatology unit (group 3, n=46) and a pediatric hepatology unit (group 4, n=25). Measuring of ceruloplasminemia (Cp), CuEXC and CuT levels was performed for all patients.

Results : Underlying diseases of the non wilsonian patients were as followed. In group 3 : ASH (n=8), NASH (n=11), HCV (n=7 with one HIV-HVC coinfected), HBV (n=8), cryptogenetic (n=8), miscellanous (n=4). In group 4 : HCV (n=5), HBV (n=3), Biliary atresia (n=4), AIH (n=1), cryptogenetic (n=7 with one fulminant hepatitis), miscellanous (n=5). Six patients of group 3 and two of group 4 had a Cp <0.16g/L, whereas one patient of group 2 had a Cp=0.19 g/L. Exchangeable copper level (N : 39-73 µg/L) was significantly higher in group 1 (mean 142 +/- 43 µg/L) compared to the other three groups : group 2 = 48+/-18 µg/L, group 3 = 66+/-19 µg/L, group 4 = 63+/-16 µg/L (p<0,05). REC >18.5% had a Se/Sp of 100% for the diagnosis of WD (group 1 vs groups 3 and 4). REC>14% had a Se/Sp of 100% for all wilsonian patients (groups 1 and 2 vs groups 3 and 4)

Conclusion : Our study confirms that exchangeable copper and particularly the determination of REC is a highly valuable tool for the diagnosis of WD.

PL-9

ROLE OF ZINC IN DEVELOPMENT: REGULATION OF STEM CELL PROLIFERATION, SURVIVAL AND DIFFERENTIATION

Cathy W. Levenson, Shannon D. Gower-Winter, Rikki S. Corniola, Deborah R. Morris, and Rohit Seth.

Department of Biomedical Sciences and Program in Neuroscience, Florida State University College of Medicine, Tallahassee, Florida, USA. cathy.levenson@med.fsu.edu

It has long been recognized that developmental zinc deficiency leads to significant growth impairment and cognitive deficits that can persist even after the deficiency has been corrected. This presentation will outline what we know about the role of zinc in physical and cognitive development as well as describe recent attempts, focused largely on stem cells, to identify the mechanisms responsible for the effects of zinc deficiency. Zinc is essential for normal stem cell proliferation, survival and differentiation. We have shown that zinc deficiency impairs proliferation of stem cells via phosphorylation and nuclear translocation of the DNA-binding transcription factor p53. Examination of downstream p53 targets revealed the regulation of 14 different genes, including cell cycle arrest genes. We have also identified two distinct molecular pathways governing apoptosis in zinc deficient neuronal stem cells. In additional to the transcriptional role of p53, we show translocation of p53 to mitochondria where it is associated with the pro-apoptotic protein BAX, loss of mitochondrial membrane potential, translocation of the mitochondrial apoptosis inducing factor (AIF) to the nucleus, and ROS production. The second pathway we identified is caspase-dependent and included activation of caspases-2, -3, -6, and -7. Targets of activated caspases include the structural protein lamin and poly-ADP ribose polymerase (PARP), which participates in DNA repair. Together these mechanisms led to chromatin condensation and DNA fragmentation, classic signs of apoptotic cell death. Finally, zinc deficiency impaired neuronal differentiation and altered binding activity of 28 transcription factors including retinoid X receptor (RXR) known to participate in the differentiation of stem cells into mature neurons. Use of a reporter gene construct containing retinoic acid response elements upstream from a luciferase gene revealed that zinc deficiency results in decreased transcriptional activity of RXR and reductions in retinoic acid-mediated gene transcription during neuronal differentiation. These results show that zinc deficiency leads to the disruptions in stem cells in the brain and other organs and implicate these mechanisms both in growth impairments and in the cognitive deficits associated with zinc deficiency.

OC-12

SERUM SELENIUM CONCENTRATIONS IN PREGNANT WOMEN IN HUNGARY: IST IT TIME FOR SUPPLEMENTATION?

Molnar J, *Hungary*

Lupeny utca 3/a. 1026 Budapest Hungary
molzsa@t-online.hu

Introduction: Selenium (Se) supplementation has been established in the treatment of certain endocrine disorders in the past few years. The Se intake of Hungarian pregnant women has not yet been determined; however, Hungary has been reported to be a country with a marginal Se deficiency. The aim of our study was to assess whether Se supplementation in Hungarian pregnant women should be taken into consideration. At present, only pregnant women with autoimmune thyroiditis are supplemented with selenomethionine in some cases.

Patients and methods: Ninety-eight pregnant women participated in the study after informed consent was obtained. They were regularly assessed during pregnancy at the 1st Department of Obstetrics and Gynecology, at Semmelweis University, Budapest, Hungary. Blood was taken in the second trimester of pregnancy from all pregnant women. Serum Se concentrations were determined by hydride generation atomic absorption spectrometry. All pregnant women were followed until delivery. It was recorded whether hypertension or pre-eclampsia developed later during pregnancy, furthermore the birth weight of the newborn and the time and method of delivery.

Results: Pregnant women had significantly lower serum Se concentrations (47.1±12.4 µg/l) than control, not pregnant women (76.4±13.2 µg/l). Serum Se concentrations varied highly in pregnant women. Altogether 9 and 7 pregnant women developed hypertension and pre-eclampsia, respectively. Those pregnant women who developed pre-eclampsia later in pregnancy had somewhat lower serum Se concentrations (40.1±11.0 µg/l) than those pregnant women who did not (48.5±12.4 µg/l).

Discussion: A general Se supplementation does not seem justified according to our results despite Se concentrations being lower in Hungarian pregnant women compared to international data. Pre-eclampsia seemed to occur more often in pregnant women with lower serum Se levels, but this has to be confirmed by a larger study.

OC-13

EFFECTS OF MATERNAL LEAD LEVELS ON PREGNANCY OUTCOMES: THE ALSPAC STUDY

CM Taylor*, J Golding, AM Emond

Centre for Child and Adolescent Health, School of Social and Community Medicine, University of Bristol, UK
**Corresponding author: caroline.m.taylor@bristol.ac.uk*
Centre for Child and Adolescent Health, School of Social and Community Medicine, University of Bristol, Oakfield House, Oakfield Grove, Bristol BS8 2BN, UK

Background/aims: The results of previous studies on the associations of prenatal blood lead levels (BLL) with pregnancy outcomes such as birthweight and pre-term delivery have been inconsistent. Our aim was to study these associations in a large cohort of mother–child pairs in the UK.

Methods: Pregnant women resident in the Avon area of the UK were enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC). Whole blood samples were collected and analysed by inductively coupled plasma dynamic reaction cell mass spectrometry (n=4285). Self-completion postal questionnaires were used to collect data on lifestyle, diet and environmental factors during pregnancy. Data collected on the infants included anthropometric variables and gestational age at delivery. Statistical analysis was carried out with SPSS v18. Regression models were adjusted for covariates including maternal height, smoking, parity and sex of the baby.

Results: The mean BLL was 3.67±1.47 (median 3.41, range 0.41–19.14) µg/dl. BLL were significantly higher in women delivering low-birthweight babies (<2500 g; 3.89±2.55 vs 3.65±1.46 µg/dl, p=0.024), but not preterm babies (<37 weeks; 3.85±1.66 vs 3.66±1.45 µg/dl, p=0.053). Increasing BLL was significantly associated with reductions in birthweight, head circumference and crown–heel length (p=0.013, p=0.028 and p=0.026, respectively). BLL ≥5 µg/ml significantly increased the risk of preterm delivery (OR 1.761, 95% CI 1.128–2.751; p=0.013) or having a low-birthweight baby (OR 1.653, 95% CI 1.013–2.969; p=0.044).

Conclusion: There was an adverse effect of BLL on pregnancy outcomes in this group of women, with reductions in birthweight, head circumference and crown–heel length, and an increased risk of preterm delivery and low birthweight, in adjusted regression models. This could have important long-term effects on the physical and neurological development of the child.

OC-14

PRENATAL EXPOSURE TO ENVIRONMENTAL MERCURY IN POPULATIONS ALONG THE COASTAL REGION OF THE INDIAN OCEAN IN SOUTH AFRICA

Kalavati Channa[a,b], Jon Ø. Odland[a], Tahira Kootbodien[c], Penny Theodorou[b], Ina Naik [b], Torkjel M. Sandanger[a,d], and Halina B. Röllin[c,e]

[a] *Institute of Community Medicine, University of Tromsø, Tromsø, Norway*
[b] *National Health Laboratory Services, NIOH, Johannesburg, South Africa*
[c] *Medical Research Council, Johannesburg, South Africa*
[d] *Norwegian Institute for Air Research (NILU), Fram Centre, Tromsø, Norway*
[e] *University of Pretoria, South Africa*
Kalavati.channa@nioh.nhls.ac.za

Mercury is a persistent heavy metal that negatively affects human health, and more importantly, the developing fetus. Many factors in South Africa, including, climate change, and poor economic, nutritional and health status, illegal gold mining, extensive coal mining as well as the release of mercury by industries all potentiate the need to investigate mercury exposure.

Study subjects included women (350) residing in three coastal areas of KwaZulu Natal who were admitted for delivery at the local hospitals. Urine from the mother and paired maternal and cord blood was collected and each participant was interviewed in the language of their choice to answer the questionnaire.

Mercury was detected in 100% of the maternal and cord blood samples. The overall results for maternal mercury levels ranged from 0.2-13 µg/l, while the corresponding cord blood levels were 0.2-18 µg/l. Site 1 (n= 100) participants had significantly higher maternal blood GM mercury (0.93 µg/l) levels than site 2 (n=200) (0.49 µg/l)) and 3 (n=50) (0.56 µg/l). The same pattern was found in the corresponding cord blood levels. Cord blood mercury in site 1 was 1.45 µg/l, site 2 was 0.7 µg/l and site 3 was 0.73 µg/l. There was a strong positive correlation (r^2 = 0.66) between maternal and cord blood mercury levels.

The percentage of subjects where the largest mercury levels in maternal blood were found above the 90th percentile were: 86% residing in site 1, 37% in the age group 20 -29, 92% with no reported environmental pollution around the home, 57% using the outdoor tap as a source of drinking water, 74% using wood for cooking, 57% consume fish once/week, 53% consuming tinned fish and 83% having their home sprayed by the malaria vector control.

Multivariate analysis revealed that maternal blood mercury levels ($p<0.001$), study site 1 ($p<0.001$) and environmental pollution in the home ($p=0.004$) and a household member is involved in fishing ($p=0.002$) were strong predictors of elevated umbilical cord mercury concentrations.

PL-10

TRACE ELEMENTS AND THYROID FUNCTION IN AGEING

Josef Köhrle

Institut für Experimentelle Endokrinologie, Charité-Universitätsmedizin Berlin, Germany; josef.koehrle@charite.de

Trace elements (TE), minerals, vitamins and hormones exert their essential biological actions at very low concentration and their status is tightly regulated along life periods and varies in health and disease. Especially, aging is associated with marked alterations of their levels measured in whole blood or its fractions, various body fluids or tissues. However, total elemental analysis provides only a rather limited insight into the biologically relevant species and their concentration. Various chemical forms of TE require speciation analysis to identify functionally active compounds or TE containing biomolecules. High public and medical interest addresses the controversial issues whether the status of individual TE is adequate for healthy aging, longevity and wellness, a prerequisite for independent and autonomous living of the elderly population. Supplementation with selected TE is required for some risk groups. Altered nutritional habits, decreased food and calorie intake, changing food preferences and nutritional imbalances in aging individuals may all lead to disturbed TE supply and status.

With respect to maintenance of adequate thyroid function, still severely impaired in several risk groups worldwide, the nutritional supply with the essential TE Iodine, Selenium and Iron is not fully secured in the ageing population. This raises the issue of recommendations for targeted supplementation. Several publications indicate prevailing mild iodine deficiency, low to inadequate Selenium intake or Iron deficiency especially in females, who are at higher risk for both benign and malignant thyroid diseases throughout life. Both thyroid cancer and autoimmune thyroid disease show increasing incidence. Whether thyroid function responds to zinc and calcium supply, which has been found to significantly decrease with advancing age and in osteoporosis, remains unclear. But increased (supplemental) Ca intake is associated with elevated risk for CVD and myocardial infarct. – Supported by DFG grants.

OC-15

EFFECTS OF STRONTIUM RANELATE ON BONES IN OSTEOPOROTIC WOMEN

Georges BOIVIN

INSERM, UMR 1033, F-69008 Lyon, France
Université de Lyon, F-69008 Lyon, France
georges.boivin@inserm.fr

Strontium ranelate (SrRan, 2g/day), a new orally effective and safe treatment for postmenopausal osteoporosis (PMOP), has demonstrated its early and sustained antifracture efficacy at the vertebral, nonvertebral and hip levels (1,2). The bone mineral density assessed by osteodensitometry is rapidly increased at both vertebral and nonvertebral levels. These results are comparable with those related for other treatments. The mechanism of action of SrRan (dissociation between bone resorption and formation) is different from that of the other treatments either purely antiresorptive (bisphosphonates, SERMs, anti-RANK ligand….) or only formative (PTH, teriparatide). In adult bone (3), the formative agents provoking an augmentation in the remodeling activity, induce a decrease in the time available for the secondary mineralization. This leads to the fact that new BSU are resorbed before they have fully completed their secondary mineralization, as proven by the presence of a large amount of uncompletely mineralized BSU and a low mean degree of mineralization (DMB). Conversely, antiresorptive agents cause a more complete secondary mineralization of BSU and provoke an increase in DMB. This was first demonstrated, using quantitative microradiography, in osteoporotic women treated for 2-3 years with alendronate (4). DMB values were significantly increased after alendronate versus placebo. The between group differences in DMB were similar to bone density measured *in vivo* at the lumbar spine level.

Intrinsic bone quality was investigated on iliac bone biopsies from osteoporotic women. At the tissue level, up to 5 years of treatment, secondary mineralization is maintained at a normal range (5,6). Strontium was heterogeneously distributed into bone tissue and exclusively present in recently formed bone. The surfaces of bone containing strontium increased with the duration. However, focal bone strontium content remained constant in the new bone (0.41±0.12 atomic %). DMB and microhardness (poster to this meeting) of the whole bone samples were maintained and distribution of DMB was heterogeneous in bone, independently of treatment duration. At the crystal level (7), incorporation of strontium within apatite crystals represents a maximum of 0.5 strontium ions per 10 calcium ions after 3 years of treatment; thus, the mean rate of substitutions of calcium by strontium is low (4.5%). Variables reflecting crystal and unit cell characteristics (crystallinity, apparent length and width/thickness of crystals, interplanar distances, and lattice parameters of unit cells) were not influenced by the treatment with SrRan.

To conclude, when compared to other treatments of osteoporosis and even after a prolonged treatment with strontium ranelate (up to 5 years), the intrinsic quality of bone was preserved, supporting the safety of this treatment at bone tissue level.

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OC-16

INTERCONNEXION BETWEEN SELENIUM, SELENOPROTEINS AND CELLULAR SENESCENCE

Yona Legrain (yona.legrain@cgm.cnrs-gif.fr), Zahia Touat (zahia.touat@cgm.cnrs-gif.fr) and Laurent Chavatte (laurent.chavatte@cgm.cnrs-gif.fr)

Selenium is an essential trace element with antioxidant properties. It is incorporated in the body as selenocysteine and forms selenoproteins. This incorporation occurs through a unique recoding mechanism of the UGA Stop codon into selenocysteine in cotranslational manner. 25 selenoproteins have been identified in humans, among them the Glutathione Peroxidases (GPx) and the thioredoxin reductases (TR) which are involved in the defence against oxidative stress. One third of these selenoproteins have unknown functions. Aging is a complex, slow, progressive and multifactorial process which leads to the body's inability to maintain its physiological balance. Studying the aging process can be done through the study of cellular senescence. Epidemiological studies of the relationship between selenium and longevity in humans have shown that in patients older than 60 years, selenium deficiency would lead to a significant reduction in longevity. We studied the interplay between selenium, selenoproteins and cellular senescence on the embryonic lung fibroblasts WI38, the historical MODEL of cellular secescence. To understand the relationship between cellular aging and selenium, we investigated the effect of selenium on various characteristics of cellular senescence (telomere length, beta-galactosidase activity, morphology…) as well as the relationship between cellular aging and selenoproteins. First, demonstrating there was a protective effect of selenium on the appearance of senescence's characteristics. Then, that cellular senescence had a selective effect on selenoproteins' expression (mRNA and protein) and UGA recoding to selenocysteine. In fine, the aim of our study is to determine which of the selenoproteins can be considered as biomarkers of cellular senescence.

PL-11

TRACE ELEMENT IMPORTANCE FOR NEUROPROTECTION AND INFLAMMATION

E Mocchegiani (Italy)

OC-17

DIABETES, TYPE II AS A NUTRITIONAL DEFICIENCY

Donald Oberleas, Ph.D.

Professor Emeritus, Texas Tech University - Lubbock, Texas

Diabetes, Type II is misunderstood and mismanaged as a chronic disease condition. It involves chromium^{+3} as a stabilizing cofactor between insulin and insulin receptor within the cell membrane. The chemical characteristics of chromium are such that it complexes with water, urea, ammonia, halides, sulfate, and particularly carboxylic acids. It's complexation with water is the most stable of any element in the periodic table at 5×10^{-7} $k_l(^{s-1})$. Since water is a prevalent compound in the body, much of the chromium is complexed with water and is carried via the GI tract as a water complex at a rapid rate. The remaining small portion of chromium, estimated at about 0.5% forms complexes with other compounds described above and is absorbed to meet the metabolic needs of the body.

Since only small amounts are needed by the body to perform its designated activities in the body that are to stabilize the insulin over the insulin receptor in a fashion that allows the insulin to function properly. There is no analytical method to measure stability because nothing happens that is measurable. This then needs the consideration that since type II, diabetes is genetic in origin, there must be some defect or substitution within the small peptide we call a chaperone which contains 4 glutamic acid residues, and two each of glycine, cysteine and aspartic acid with a molecular weight of ~1500 and contains 4 chromium^{+3} most likely complexed with the glutamic acid residues. Insulin also contains 4 glutamic acid residues. Probably the insulin receptors also contain 4 glutamic acid residues.

Though the numbers of subjects are small, there is evidence that following the intravenous injection of ^{51}Chromium to human subjects shows a more rapid disappearance of ^{51}Chromium from the blood and a more rapid excretion via the urine. Also by ingesting 200 µg of chromium twice each day is an effective therapy for type II diabetics without drug therapy. Numbers of individuals are currently small but this therapy is very effective.

OC-18

HEAVY METALS AND MULTIPLE SCLEROSIS

*Palazón-Bru I, **Varadé J, *López Lozano L, *González- Estecha M, **Urcelay E, ***Arroyo R, *Arroyo M.

**Laboratory Medicine Department. Trace Element Unit*
***Inmunology Department*
**** Neurology Department.*
Fundación para la Investigación Biomédica, Hospital Clínico San Carlos.
Madrid (Spain) - irene_pb@hotmail.es

Background. Multiple sclerosis (MS) is a common disease of the central nervous system, with genetic and environmental factors involved in its development. Recently, a postmortem study on children and young adults showed that lifelong exposure to air-pollution is associated with neuroinflammation, altered innate immune responses and disruption of the blood-brain barrier. Heavy metals have been proposed as a possible cause of neurodegenerative diseases such as MS. rs4613763 maps next to a prostaglandin receptor gene PTGER4, a region associated with MS susceptibility. Prostaglandin secretion was previously related with Pb2+ exposure through the activation of phospolipase A2 and COX2. The polymorphism rs1520333 lies at the PRKACN1-ZC2HC1A-IL7 region also associated with MS risk.

Our aim was to investigate the impact of lead, cadmium and mercury levels on MS progression and their association with the main MS genetic risk factors identified by genome-wide association studies.

Material and methods. We studied 90 MS patients (65.5% female) with a mean age of 37.34 (SD 9.16) and 90 healthy controls (67.8% female) with a mean age of 37.50 (SD 8.97) matched by age and gender at blood extraction. Mercury concentration in blood was measured by atomic absorption spectrometry and thermal decomposition amalgamation. Lead and cadmium concentrations in blood were measured by electrothermal atomic absorption spectrometry.

Results. Lead concentration in blood showed a non significant difference between patients and controls [p=0.08; median (IQR)=1.55 µg/dL(0.67-2.52) vs 1.10 µg/dL(0.70-1.90)]. Cadmium and mercury blood levels did not evidence significant differences. Higher lead levels in blood were associated with rs4613763 T allele (p=0.01; OR=3.70 95%CI 1.07-14.05) and with rs1520333 C allele (p=0.006; OR=2.80 95%CI 1.35-5.86).

Conclusions. Lead levels in blood associate with polymorphisms involved in MS susceptibility. Further studies are necessary to confirm these results.

PL-12

TRACE ELEMENTS NEEDS AND DEFICIENCIES IN PETS

Professor Dominique Grandjean, DVM, PhD, HDR

Alfort School of Veterinary Medicine, University Paris East, Maisons-Alfort (France)
dgrandjean@vet-alfort.fr

The ideal diets for animals such as dogs and cats contain optimal ranges of essential nutrients (around 50) for specific purposes. The ranges may be broad for the indolent, indoor pet, but become more narrow as demands for performance, or to cope with stress or pathologies, increase. Such times might include physiological periods of the life cycle i.e. breeding, growth periods, showing, hunting, working or sorting, or aging.

The paradox of the sedentary dog or cat is that it apparently does well on any nondescript diet, but, when stress situation occurs, the animal lacks reserves and cannot cope. An animalthat has been fed on a minimal diet will be vulnerable to heat or cold, infection or trauma ; it twill be negatively affected by changes in environment, routine, or expectations. It can even reach the point where clinical signs of deficiency in micronutrients like trace elements can occur,such as in what is called by veterinarians the « generic food disease ».

The essential trace elements in dogs and cats include iodine, iron, manganese, copper, molybdenum, zinc, selenium, fluorine, cobalt and chromium. Other candidates for recognition as essential trace elements could be silicon, arsenic and vanadium. They occur within limited ranges of concentrations at specified sites in the body, and must have known specific biochemical functions. These characteristics distinguish them from adventitious elements, such as lead and cadmium or mercury.

The actual ranges of contents of trace elements in petfoods are extremely broad.It may be assumed, for the sake of arguments, that the manufacturers know the bioavailibilities of the trace elements in their products. Then, the trace elements contents of products might result from application of these bioavailibilities to recommandations for minimum requirements of available nutrients...

Multiple deficiencies of trace elements usually attend only three types of petfoods. Two represent the extremes of energy density ; one contains excessive fat and the other contains excessive fiber and phytin. The third type is high in calcium. Therefor the use of mineral or organic salts with a high bioavailibility is important, and practical recommandations should adress the minimum requirements of « available » trace elements. In practice however, we must admit that formulation and evaluation of petfoods must include the understanding that these minimums may not be well determined yet, that bioavailibilities are subject to many conditionning factors, and that minerals ostensibly selected to supply the essential trace elements are chosen for more reasons that single purpose.

PL-13

BIOLOGICAL EFFECTS CONNECTED WITH METAL NANOPARTICLES ENTRY INTO ORGANISM

E. Sizova, Orenburg State University, Orenburg, Russia e-mail: Sizova.L78@yandex.ru, S. Miroshnikov, Orenburg State University, Orenburg, Russia: inst_bioelement@mail.ru, V. Polyakova, Orenburg State Medical Academy of Federal Agency in Public Health and Social Development, Orenburg, Russia, N. Gluschenko, Institute of Energy Problems in Chemical Physics RAS, Moscow, Russia.nnglu@mail.ru

A. Skalny Institute of Bioelementology, Orenburg state university, Orenburg, Russia, inst_bioelement@mail.ru

There was carried out a research on biological effects of copper and iron at their entry into organism of animals on the example of structural and functional reorganization of main target organs such as liver, kidneys, spleen, brain, and apoptosis intensity.

The research was conducted on male Wistar rats with weight 150-180 g. Animals were weekly intramuscularly injected with aqueous suspension of copper nanoparticles in dosage 2.0 mg/kg of animal weight. Test samples were taken 3 hours, 1 day, 3 and 7 days after each injection. Caspase-3 expression was assessed to reveal readiness to apoptosis. Immunohistochemical researches were carried out with monoclonal antibodies on paraffin slices using Bio Genex Super Sensytive Detection System (USA) and protocols of the producer. Immunopositive cells were counted among 1000 and expressed in ‰. It is established that copper nanoparticles distribute in organs and tissues of animals and cause specific structural changes. The increase of copper nanoparticles in the organism up to toxic threshold (maximum tolerated dose) results in dystrophy and tissue necrosis.

Statistically significant enhancement of Caspase 3 expression in microgliocytes (brain macrophages) has been registered after the dose 2 mg/kg of animal body weight, in liver cells – after total dose 6 mg/kg of animal body weight, in proximal kidney tubules - after total dose 6 mg/kg of, in spleen cells – after total dose 24 mg/kg.

The most significant morphometric changes of white pulp of spleen occur in 7 days after repeated introduction. Lymphoid follicles increase after the second introduction as a result of germinal center and mantle enhancement, marginal layers where the processes of cell differentiation and outcome into red pulp occur. Received data enables us to propose using index of cells readiness to apoptosis defined by Caspase 3 expression as an assessment criterion of copper nanoparticles safe introduction. Copper nanoparticles in dose of 2 mg/kg express neurotoxity. Hepatoxicity and nephrotoxicity of copper nanoparticles is expressed in dose 6 mg/kg. Splenic lymph follicles enhance caspase-3 expression in response to copper nanoparticles introduction in dose of 24 mg/kg, i.e. In dose close to LD_{100}. The received data attest to high biological potency of copper nanoparticles comparing to iron nanoparticles and enable to define copper particles as modulators of apoptosis in organism. It's proposed to use the index of cells readiness to apoptosis defined by caspase-3 expression as

criterion for assessment of safe nanoparticles introduction in organism. The quality of copper nanoparticles to enhance cell readiness to apoptosis in nontoxic doses (2-6 mg/kg) established by us may be used in chemotherapy of malignant neoplasms where apoptosis of mutant cells is being inhibited. Moreover, the conducted research makes it obvious that the index of cells readiness to apoptosis express the beginning of irreversible structural changes regardless of tissue specific character in response to copper introduction. By this index we can judge on tolerance of metal introduced, target organs, find optimal and safe methods of nanoparticles introduction into the organism for their further use in composition of medicines.

OC-19

DETERMINATION OF INORGANIC ELEMENTS IN BLOOD OF GOLDEN RETRIEVER MUSCULAR DYSTROPHY DOGS USING NEUTRON ACTIVATION ANALYSIS

S. Metairon[1], C.B. Zamboni[1], M.F. Suzuki[1], C.R. Bueno Jr[2], H.V.A. Caetano[2], M. Zatz[2]

1 *Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN/SP - Av. Professor Lineu Prestes 2242 05508-000, São Paulo, SP, Brazil - metairon@live.com*

2 *Centro de Estudos do Genoma Humano, Instituto de Biociências, USP - Rua do Matão, Travessa 13106 - 05508-090, São Paulo, SP, Brazil - carma011@yahoo.com.br*

In recent years, the Nuclear Structure Laboratory at IPEN – CNEN/SP (Brazil) has performed measurements related to the determination of inorganic elements in blood of animals used for experimentation (rats, rabbits, mice) using Neutron Activation Analysis technique (NAA). The advantage in using NAA to investigate blood is related to the fact that this analytic technique uses small quantity of blood (10 to 100 µL) when compared with the conventional clinical practices performed in serum (0.5 to 1.0 mL), resulting in an efficiency procedure for biochemical analysis mainly when the biological material is scarce. The success in these applications, with small and medium-sized animal models, has motivated us to study in more details the anomalies caused by Duchenne Muscular Dystrophy (DMD), an illness of hereditary character that affects approximately 1 in every 3,600 to 6,000 live male births in the world. Nowadays, many promising therapeutic strategies have been developed in animal models with DMD. An animal model which has a phenotype similar to patients with DMD has been bred in Brazil: Golden Retriever Muscular Dystrophy dogs (GRMD). In these dogs, muscle degeneration and fibrosis are predominating, leading to a progressive loss of structure and muscle function, and resembling a human pathogenesis. In this study, elements of clinical relevance (Ca, Cl, Mg, K, Na) were determined in blood of the GRMD using NAA at the IEA - R1 nuclear reactor. These data may help to evaluate the efficiency of new treatments as well as to compare the advantages of different treatment schedules before performing tests in patients with DMD.

OC-20

EFFECTS OF TIO_2 NANOPARTICLES ON EMOTIONAL BEHAVIOR AND BIOCHEMICAL PARAMETERS IN ADULT WISTAR RATS

Salem Amara[1], Wahid khemissi[1], Imen Mrad[1], Naima Rihane[1], Imen Ben Slama[1], Lassaad El Mir[2,3], Mustapha Jeljeli[1], Khemais Ben Rhouma[1], Hafedh Abdelmelek[1] and Mohsen Sakly[1].

1 Laboratoire de Physiologie Intégrée Faculté des Sciences de Bizerte, 7021 Jarzouna, Tunisia
2 Laboratory of Physics of Materials and Nanomaterials applied at environment, College of Sciences in Gabes.
3 Al-Imam Mohammad Ibn Saud Islamic University, College of Sciences, Department of Physics, Riyadh 11623, Saudi Arabia.
Corresponding Author: Dr Salem AMARA
Laboratoire de Physiologie Intégrée, Faculté des Sciences de Bizerte, Jarzouna 7021, Tunisia
amara_salem_fsb@yahoo.fr

The rapidly developing field of nanotechnology is becoming a potential source for human exposure to nanoparticles. Titanium dioxide (TiO_2) nanoparticles have been widely produced in industrial processes for several years. The aim of this study was to investigate the effects of TiO_2 nanoparticles on plasmatic biochemical parameters and the emotional behavior in adult Wistar rats. Rats were treated by intraperitoneal injection of TiO_2 nanoparticles (20-30 nm) at a dose of 25 mg/kg. For toxicity evaluation of nanoparticles sample, body weight, organ coefficient, blood biochemistry panel assay (AST, ALT, LDH, uric acid, creatinine, and glucose content) and emotional behavior parameters were determined. Sub-acute TiO_2 nanoparticles treatment decreased the body weight, but increased the relative brain weight. Biochemical assessment in plasma samples showed that TiO_2 nanoparticles injection increased uric acid concentration and AST activity in rats. However, the same treatment decreased the creatinine level, but had no effect on glucose concentration, ALT and LDH activity. The emotional behavior of control and treated rats was tested in elevated plus-maze. Interestingly, our results showed that TiO_2-treated rats spent more time in the secured closed arms and entered the anxiogenic open arms less frequently than control.

Our results suggest that TiO_2 nanoparticles intoxication could alter biochemical parameters related to changes in organ function and leads to emotional behavior impairment of rats.

OC-21

EFFECT OF A NEWLY SYNTHESIZED MANGANESE SALT ON THE EIMERIA TENELLA (PROTOZOA) EXPERIMENTALLY INFECTED BROILER CHICKENS

Milena Anissimova[1], Venzislav Koinarski[2], Delka Salkova[1], Veselin Nanev[1], Margarita Gabrashanska[1]

1 *Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria - m.gabrashanska@gmail.com*
2 *Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria*

The study was undertaken to determine the dietary supplementation with manganese complex compound (2Gly.MnCl2.2H2O) on the mortality, body weight, oocyst and lesion index, the antioxidant defense system and serum and liver Mn level in broiler chickens experimentally infected with Eimeria tenella. Clinically healthy broiler chickens, Cobb 500 hybrids, were divided into 3 groups: 1st gr. – control (untreated and non-infected), 2nd gr. – infected with 8.104 sporulated oocyst of E. tenella and 3rd gr. – infected with with 8.104 sporulated oocyst of E. tenella and received 2Gly.MnCl2.2H2O in the food. On 7th day post infection liver and serum samples were collected for biochemical analyses – (determination of vitamin A, C and E, enzyme activity of superoxide dismutase and catalase, and malondialdehyde) and Mn determination, and ceca - for lesions and oocyst index estimation. Biochemical, chemical and parasitological methods were applied in the study. Body weights and mortality were checked on 1st and 7th day post infection. Antioxidant imbalance was developed in the infected and treated and untreated chickens. Hypovitaminoses A, B and C, reduced Mn level, increased MDA, and deviation in the activity of SOD and CAT were observed in the infected groups. Mn supplementation reduced oocyst index, increased body weights, reduced mortality and improved antioxidant system in the infected chickens. Diet complementation with Mn compound could be beneficial for the infected hosts in ameliorating their performance and defense system.

OC-22

LONG-TERM STUDY OF OVINE PULMONARY ADENOCARCINOGENESIS IN SHEEP WITH MARGINAL VS. SUFFICIENT NUTRITIONAL SELENIUM SUPPLY: RESULTS FROM COMPUTED TOMOGRAPHY, PATHOLOGY, IMMUNOHISTOCHEMISTRY, JSRV-PCR AND LUNG BIOCHEMISTRY

Esther Humann-Ziehank*a**, Kostja Renkob, Michael Ludwig Bruegmannc, Vermuri Rama Devid, Marion Hewicker-Traustweind, Arnim Andreaea, Martin Ganteraa

a *Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, D-30173 Hannover, Germany*
b *Institut für Endokrinologie, Charité Universitätsmedizin Berlin, D-13353 Berlin, Germany*
c *Lower Saxony State Office for Consumer Protection and Food Safety, Veterinary Institute Oldenburg, Dept. of Pathology, Germany*
d *Department of Pathology, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, D-30173 Hannover, Germany*
** Corresponding author. Tel. ++49 511 856 7260; fax ++49 511 856 7684*
esther.humann@tiho-hannover.de

The impact of selenium (Se) in carcinogenesis is still debatable due to inconsistent results of observational studies, recent suspicion of diabetic side effects and e.g. dual roles of glutathione peroxidases (GPx). Previously, our group introduced long-term studies on lung carcinogenesis using the Jaagtsiekte sheep retrovirus (JSRV) induced ovine pulmonary adenocarcinoma (OPA) as an innovative animal model. The present report describes the results of sufficient (0.2 mg Se/kg dry weight (dw)) vs. marginal (<0.05 mg Se/kg dw) nutritional Se supply on cancer progression over a two-year period in 16 animals. Computed tomography (CT) evaluation of lung cancer progression, final pathological examination, evidence of pro-viral JSRV-DNA in lung, lymph nodes and broncho-alveolar lavage cells as well as biochemical analysis of Se, GPx1 and thioredoxin reductase (TrxR) activity in lung tissue were recorded. Additionally, immunohistochemical determination of GPx1 expression in unaffected and neoplastic lung cells was implemented.

The feeding regime caused significant differences in Se concentration and GPx1 activity in lung tissue between groups, whereas TrxR activity remained unaffected. JSRV was evident in broncho-alveolar lavage cells, lung tissue and lung lymph nodes. Quarterly executed CT could not demonstrate differences in lung cancer proliferation intensity. Necropsy and histopathology substantiated CT findings. Immunohistochemical analysis of GPx1 in lung tissue suggested a coherency of GPx1 immunolabelling intensity in dependence of tumour size. It was concluded that the MODEL proved to be suitable for long-term studies of lung cancer proliferation including the impact of modifiable nutritional factors. Proliferation of OPA was unaffected by marginal vs. sufficient nutritional Se supply.

PL-14

TRACE ELEMENTS IN PHOTOPROTECTION

Pr MT Leccia

Clinique de Dermatologie, Allergologie et Photobiologie
CHU Albert Michallon, Grenoble

Exposing the skin to sunlight leads to DNA damage and photochemical generation of reactive oxygen species (ROS) implicated in skin carcinogenesis and skin aging. To neutralize ROS and maintain their integrity, skin cells are provided of various defense mechanisms including antioxidant enzymes such as selenium(Se)-dependent glutathione peroxidases and copper(Cu)-zinc(Zn) superoxide dismutases. Optimizing enzyme activities with Se and Zn supplementation provide extra benefit in culture models and in animals, especially under oxidative stress from excessive UVA exposure. In addition to their antioxidant role, Se and Zn also demonstrate interesting activities on DNA repair. Despite encouraging preclinical studies, results from topical and/or oral supplementations in humans remain disappointing. Current studies lead to recommend use of association of antioxidant molecules to optimize the natural antioxidant balance and to be careful with supplementation in patients who have a good antioxidant status and potential precarcinogenic tumors. The question of a beneficial role of antioxidant molecules (such as Se or Zn) adjunction in sunscreens is still opened.

OC-23

EVALUATION OF AGE IMPACT ON THE PROTECTIVE EFFECT OF SELENIUM AGAINST UVA IRRADIATION IN PRIMARY HUMAN KERATINOCYTES

W Rachidi[1*], I Hininger[2], D Beal[1], M Osman[2], C Favrot[1], MT Leccia[3], E Blouin[4] and AM Roussel[2]

[1] *Grenoble University, CEA, Nucleic Acids Lesions laboratory, Grenoble, France. *E-mail: walid.rachidi@ujf-grenoble.fr*
[2] *Grenoble University, INSERM U1055, Laboratoire de Bioénergétique Fondamentale et Appliquée, Grenoble, France*
[3] *Department of Dermatology and Venereology, University Hospital Albert Michallon, Grenoble, France*
[4] *Labcatal Pharmaceuticals, Montrouge, France*

Background Few studies have focused on the protective role of selenium (Se) on skin aging and photoaging although selenoproteins are essential for keratinocyte function and skin development. Previous data are controversial mainly due to difficulties in assessing chronic multifactorial effects on aging. However, experimental evidence on cell cultures (keratinocytes or fibroblasts) has showed that Se supplementation might be beneficial against several oxidants, pollutants or exposure to UV.

To our knowledge, the impact of Se supplementation on skin cells obtained from elderly and young donors has not been reported. So, the main objective of our work was to evaluate the effects of Se supplementation (as its inorganic form sodium selenite (NaSe)) on skin keratinocytes at baseline or after exposure to UVA irradiation

Methods Keratinocytes were obtained from normal skin biopsies of elderly (60-70 years old) or young (20-30 years old) donors and were pre-treated with 30 or 240 nM of NaSe for 72h, followed or not by different doses of UVA. Se concentrations were determined by ICP-MS, cell survival was examined by using both MTT and clonogenic cell survival assay and DNA damage induction and repair were evaluated by the alkaline Comet assay and by mass spectrometry (LC-MS/MS).

Important results We showed that NaSe has better uptake by keratinocytes obtained from young donors compared to elderly ones. Low doses of NaSe (≤30 nM) were very potent protector against UVA-induced cytotoxicity and genotoxicity on young keratinocytes, whereas the protection efficiency of NaSe on old keratinocytes was obtained only at higher concentrations (≥ 240 nM). These original data strongly suggest an increased vulnerability of keratinocytes with age against photoaging and should be taken into account regarding Se needs in elderly. The mechanisms by which NaSe could protect against photoaging are still unclear and the proteomic approach that we are performing should help us to propose an hypothesis.

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OC-24

COPPER DEFICIENCY - A NEW REASON OF ANDROGENETIC ALOPECIA?

Skalnaya M.G.

NGO Centre for Biotic Medicine, Moscow, Russia
E-mail: skalnaya@yandex.ru

Previously (Skalnaya, 2010) we described the statistically significant decreasing of Cu content in temporal hair samples compared to occipital ones in premenopausal women suffering from androgenetic alopecia (AA). Administration of Cu aspartate (4-6 mg daily, per os) during 3-6 months demonstrated moderate clinical effectiveness in therapy of AA in women. In this study we investigated premenopausal women (20-43 y/o), suffering from AA (n=45, AA group), and 56 women without AA (control group). All women did not receive HRT. The occipital hair and serum samples were analyzed by ICP-MS. It was found no statistically significant difference in macro and trace elements between the groups, except Si (lower in AA group: 27.9±3.5 vs 32.5±2.3 ppm, P<0.05). Cu content had only a tendency to decreasing (17.7±1.2 vs 19.1±1.2, P<0.01). The determination of serum Cu revealed its decreasing in AA women (0.86±0.02 vs 1.04±0.04 ppm, P<0,001). The similar picture was observed in group of 48 men (19-39 y.o.). In both sexes the simultaneous decreasing of serum Cu concentration and coeruloplasmin (CP) level were obtained. In case of androgens excess and/or elevated androgen/estrogen ratio, also in patients with dominated estrogen in peripheral blood, the decreased Cu concentration in serum was revealed. **Conclusion**: 1) recent study suggests the hypothesis about possible pathogenetic role of Cu deficiency in AA both in women and men. 2) in women with AA the lower serum Cu corresponds to lower CP; this suggest the diminishing of Cu deposits in liver. 3) the lower serum Cu level in AA women is independent from different sexual hormones dominating. 4) Cu replacement therapy can be more clinically effective than HRT in women with AA. 5) we suppose that Cu deficiency regardless of its origin can cause hair loss in women and men because of Cu role in regulation of epithelial growth factor, lysil oxidase activity (collagen and elastin synthesis), vascularization and hair follicle cycling.

PL-15

HEAVY METAL AND PUBLIC HEALTH ISSUES: ENVIRONMENT AND CHILDHOOD (INFANCIA Y MEDIO AMBIENTE) PROJECT: NEURODEVELOPMENT QUESTIONS AND MERCURY.

J Ibarluzea[1, 2]; Ferran[1,3] B; Fernandez M[1,4]; Tardón A[1,5]; Torrent M[1,6]; Vioque J[1,7]; Sunyer J[1,8].

1 CIBER Epidemiologia y Salud Pública,
2 Subdirección Salud Pública de Gipuzkoa, Basque Country.
3 Centre for Public Health Research. Generalitat Valencia
4 Universidad de Granada
5 Area de Med. Prev. y Salud Publica. Universidad de Oviedo.
5 Area de Salut de Menorca
6 Universidad Miguel Hernadez
7 Centre for Research in Environmental Epidemiology

The INMA—INfancia y Medio Ambiente—(Environment and Childhood) Project is a prospective population-based cohort study carried out in Spain and based on a network of birth cohorts that aim to study the role of environmental pollutants in air, water and diet during pregnancy and early childhood in relation to child growth and development from early foetal life until adolescence. INMA was based on the experiences acquired by three previous birth cohorts: Ribera d'Ebre, Menorca and Granada and the new four birth cohorts: Valencia, Sabadell, Asturias and Gipuzkoa.

Pre-natal and early life exposures are associated with child development and health and predispose to late adult effects. One of the specific aims of the project is to evaluate the internal dose of some neurotoxic metals like mercury and lead on neurodevelopment. These chemicals may lead to adverse outcomes as a consequence of their early action.

Hg cord blood levels and dietary fish intakes during pregnancy were studied. Results showed that: Maternal daily seafood consumption was 78g/d and the geometric mean for T-Hg was 8.2µg/L and that doubling in consumption of large oily fish was associated with an increase of 11.4% (3.8 to 19.6%) in cord blood T-Hg levels, of 8.4% (95%CI: 5.7 to 11.2%) in the case of canned tuna and 8.3% (95%CI: 5.5 to 11.1%) in that of lean fish. The proportion of children with levels higher than those established by USEPA to protect child development was 64%.Stratified findings by sex suggest a negative association between prenatal exposure to total mercury and psychomotor development among female infants (β = −1.09, 95% CI: −2.21, 0.03). Nevertheless, maternal fish intakes of >2–3 times/week, among children breast-fed for <6 months, were associated with significantly higher scores on several MCSA subscales compared with intakes ≤1 time/week.

There is a need to analyze the association between the different areas of cognitive and psychomotor development at 4 years age.

OC-25

USE OF OXIDATIVE STRESS BIOMARKERS IN SALARIA BASILISCA FOR ASSESSING HEAVY METALS POLLUTION IN THE GULF OF GABES

Sana Barhoumi[(ac)], Imed Messaoudi[(b)], Mohamed Tahar Sfar [(a)], Abdelhamid Kerkeni [(a).]

Unité de recherche: Eléments trace, radicaux libres, systèmes antioxydants et pathologies humaines et environnement. Laboratoire de biophysique. Faculté de médecine de Monastir, Tunisia.
Unité de recherche: Génétique, biodiversité et valorisation des bioressources. Institut supérieur de biotechnologie de Monastir, Tunisia.
sanabarhoumi20@yahoo.fr

In the present work, we investigated the potential use of several oxidative stress biomarkers in Salaria basilisca for assessing heavy metals pollution in the Gulf of Gabes. For this purpose, variations due to spatial and seasonal factors were considered. Fishes were collected during the four seasons between November 2007 and August 2008 from heavy metals polluted (S1) and unpolluted (S2) sites in the Gulf of Gabes. A set of parameters were measured in the liver such as Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase (CAT) activities; Glutathione (GSH) and Malondialdehyde (MDA) levels. Results showed that all parameters presented in summer a significant increase ($p < 0.001$) in both S1 and S2. Also, a significant increase ($p<0.001$) was noted for GSH and MDA levels in autumn in S1. The utility of each biomarker is discussed in the context of the roles and advantages of selecting a battery of antioxidant defense biomarkers and seasons for detecting pollution problems.

OC-26

THE PROTECTIVE EFFECTS OF ORGANIC AND INORGANIC SELENIUM AGAINST THE OXIDANT POTENTIAL OF MONOCYCLIC AROMATIC AMINES IN DIFFERENT CELLULAR FRACTIONS

Pinar Erkekoglu[1,2], Ming Wei Chao[1], Wenjie Ye[1], Laura Trudel[1], Jing Ge[1], Bevin P. Engelward[1], Belma Kocer Giray[2], Gerald Wogan[1], Steven R. Tannenbaum[1,*]

[1]*MIT Biological Engineering Department, Cambridge, Boston, USA*
[2]*Hacettepe University Faculty of Pharmacy, Department of Toxicology, Ankara, TURKEY*
**Correspondence: erkekp@mit.edu*

Monocyclic aromatic amines (MAAs) are a group of chemicals that are ubiquitous in the environment. Most individuals experience lifelong exposure to these compounds from several sources, occupational exposure or *via* tobacco smoke. 3,5-dimethylaniline (3,5-DMA) is associated with bladder cancer incidence in U.S. This study was designed to investigate the possible protective effect of selenocompounds (sodium selenite at 10 nM, selenomethionine at 30 μM) against the cytotoxic and genotoxic properties of 3,5-dimethylaminophenol (3,5-DMAP), the metabolite for 3,5-DMA, in CHO cells. 3,5-DMAP (25 μM) caused a dose-dependent increase in both cytotoxicity and reactive oxygen species (ROS) production. Besides, significant elevations in the activities of both cytoplasmic and nuclear antioxidant enzymes [i.e. glutathione peroxidase, thioredoxin reductase, catalase, glutathione reductase] and marked decreases in superoxide dismutase activity (100%) were determined. Elevations in both cytoplasmic (300%) and in nuclear (450%) oxidized glutathione levels along with significant enhancements in lipid peroxidation levels (200% in cytoplasm, 765% in nucleus) were also observed. Protein oxidation significantly increased in both cytoplasm (70%) and nucleus (55%). Besides, both tail moment and %tail DNA increased significantly in Comet assay. Selenocompounds were found be protective against the toxic effects of 3,5-DMAP. The results demonstrated that one of the mechanisms underlying the toxicity of 3,5-DMAP is the change in oxidant/antioxidant balance in different cellular fractions and selenocompounds are protective against its cytoplasmic and nuclear toxicity.

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OC-27

STUDY OF SERUM CERIUM AND ITS RELATIONSHIP WITH ATMOSPHERIC POLLUTION CAUSED BY SUSPENDED PARTICULATE MATTER (PM2.5) IN MADRID

González-Estecha M*, Aránguez E**, Höllriegl V***, Trasobares E*, Fuentes M*, Herráiz MA*, Michalke B***.

**Laboratory Medicine Department. Trace Element Unit. Hospital Clínico San Carlos. Madrid (España). Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC). Madrid (Spain).*
*** Consejería de Sanidad. Comunidad de Madrid*
****Institute of Radiation Protection and Research Unit Analytical BioGeoChemistry. Helmholtz Zentrum München*
Email: montse@cmpx.net

Background. The general population is increasingly exposed to cerium (Ce), due to its use in catalyzers and as a diesel additive. Some studies have suggested a link between elevated Ce levels and endomyocardial fibrosis and acute myocardial infarction. Exposure to suspended particulate matter PM2.5, originating mainly from combustion, increases the risk of cardiovascular and respiratory diseases. The objective of this study was to determine serum cerium levels in a sample of women from Madrid (Spain) and analyze the short term relationship between these levels and exposure to PM2.5.

Material and Methods. Twenty-six women were recruited in Madrid in 2009. The cerium in serum (ng/L) was determined by inductively coupled-plasma mass spectrometry in a Perkin Elmer ICP-MS ELAN DRC II. The PM2.5 levels expressed in µg/m3 of air were those registered at environmental monitoring stations in the region of Madrid, which apply a special prediction MODEL (kriging).

Results. The mean level of cerium in serum was 38.8 ng/L SD=11.4. A positive correlation was observed between serum cerium levels and model-predicted PM2.5 levels in the area of residence of each woman for the day before the blood draw ($r=0.508$ $p=0.008$), for the previous three days ($r=0.755$ $p<0.001$) and the seven days prior to the blood draw ($r=0.751$ $p<0.001$). No correlation was detected for the day of the blood draw.

Conclusions. The Spanish women in the study presented serum cerium levels higher than those referred in other countries, probably due to the higher atmospheric pollution in Madrid. A statistically significant, strongly positive correlation was observed between the serum cerium levels of the women in Madrid and the mean and maximum levels of PM2.5 the day before the blood draw, as well as three and seven days before the blood draw. More studies would be necessary to help clarify the relationship between cerium and PM2.5, as well as their possible impact on health.

Poster Abstracts

1. Nutrition

1-1

CURRENT PERSPECTIVES ON ZINC NUTRITION IN SOUTH ASIAN DEVELOPING COUNTRIES

Saeed Akhtar

Department of Food Science and Technology
Bahauddin Zakariya University Multan
Email: saeedbzu@yahoo.com

Zinc deficiency is highly prevalent in a major part of the world and is considered a risk factor for morbidity and mortality. Predictably, half of the global population is at risk of low intakes of zinc. Prevalence of zinc deficiency in developing countries is much higher and the situation in South Asia is exceedingly precarious and appears to deleteriously affect the populations especially from poorer settings. In addition to a few important underlying determinants like low dietary intake, low bioavailability, hygiene and recurrent infections, there are several other factors including socio economic status, political situation and priority setting of national agenda that limit the possibilities to overcome the risk of zinc deficiency in India, Pakistan, Sri Lanka, Bangladesh and Nepal. Reliable and national level data are not widely available to clearly demonstrate the extent of the prevalence of zinc deficiency; however numerous small studies highlight the likelihood of widespread marginal zinc deficiency among children and pregnant women in these regions. Magnitude of micronutrient deficiencies including zinc in Pakistan is high enough to suggest immediate remedial measures. Estimates show high extent of zinc deficiency among children and pregnant women in Bangladesh. Virtually, no holistic study or national survey typically focusing zinc nutrition assessment of the extent of zinc deficiency in Sri Lanka and Nepal was carried out in the recent past. Diarrhoea, respiratory infections and malaria have been associated with zinc deficiency particularly in low income developing countries. Supplementation, fortification and dietary diversification are the most viable strategies to enhance zinc status among various population groups. Conclusively, the information provided in this review may not only help local governments to scale up policies and strategies to control the issue but also the international organizations to clearly comprehend and identify the areas for nutritional improvement in South Asia.

1-2

THE EFFECT OF CHROMIUM PROPIONATE ON THE HUMORAL IMMUNE RESPONSE AND PERFORMANCE OF BROILERS AGAINST NEWCASTLE DISEASE IN THE TROPICS

E. Didacus

Department of Veterinary Pathology and Microbiology University of Nigeria, Nsukka

Chromium (Cr) has been considered as an essential micronutrient for humans and animals by nutritionists. The primary role of Cr in metabolism is in enhancing the glucose uptake by the cells. Cr also activates certain enzymes and stabilizes proteins and nucleic acids. Its supplementation reduces the negative effects of environmental stress. Newcastle Disease (ND) virus potentially infects most species of birds, and for susceptible poultry it is highly contagious and usually fatal. In rural Africa, the predominant chicken production systems (backyard farms) are based on indigenous domestic fowl (*Gallus gallus domesticus*), and ND is rated as the most devastating disease in these farms. The results of the humoral immune responses indicated that total antibody titers to Newcastle disease vaccines were much higher in groups I, III, and V chicks that received Cr-Prop when compared to groups II and IV that not received Cr-Prop. The results of the humoral immune responses of the Cr-Prop treated, NDV vaccinated and IBD vaccinated groups I (2.1) was higher than the untreated, NDV unvaccinated and IBD vaccinated group II was (1.9) while the Ce-Prop treated NDV vaccinated and IBD vaccinated group III (2.3) was higher than the untreated, NDV unvaccinated and IBD vaccinated group IV had (1.3). The Cr-Prop treated, NDV vaccinated and IBD vaccinated groups V had (2.5). Cr-Prop supplementation increased significantly ($P<0.05$) body weights of the broilers. Groups I (3.23Kg) was significantly ($P<0.05$) higher than group II (2.55Kg) while group III (3.13Kg) was higher than group IV (2.62Kg); group V had (3.29 Kg). The weekly mean body weight gain of the treated groups I, III and V at the end of the study were significantly ($p \geq 0.05$) higher than the untreated groups II and IV. The feed conversion ratio revealed variable significant ($p \geq 0.05$) differences which did not reflect dietary inclusion of organic Cr-Prop. Therefore, Cr-Prop supplementation improved the immune response to ND vaccine and the performance of broiler such as live body weight, body weight gain, feed intake and feed conversion ratio in broilers.

1-3

INVESTIGATION OF SULFUR IN BLOOD: A NECESSITY FOR THE BRAZILIAN POPULATION

Laura C. Oliveira, Cibele B. Zamboni, Sabrina Metairon

Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN/SP
Av. Professor Lineu Prestes 2242 - 05508-000, São Paulo, SP, Brazil
laura@ipen.br, czamboni@ipen.br, metairon@usp.br

The S in the body participates in the repair and construction of tissues and cells, in the formation of several vitamins and proteins and also helps to combat the microbes and parasites. The amount of S required per day is unknown to the general population, but the recommended value, from United States Department of Agriculture (Nutrient Database Laboratory), ranges from a minimum of 100 mg to a maximum of 850 mg. Also for Brazilian population the intake of S is not well established but, the interest in its evaluation becomes relevant due to the use of the elemental-S as a fertilizer in soils as well as the use of sulfate salts as a growth-promoting feed additives for farm animals (chickens, turkeys and pigs) highly consumed by the Brazilian population. Another aspect to be considered is the intake of some grains rich in S (mainly rice and beans) and dry fruit (that contain sulfur dioxide for keeping the color and protecting the flavor from oxidation) which is also present in Brazilian diet.

In this study the NAA technique was applied to determine S in whole blood from Brazilian habitants for the proposition of an indicative interval. The measures were performed considering lifestyle factors (non-smokers, non-drinkers and no history of toxicological exposure). The influence of age was also investigated considering several ranges (18-29, 30-40, 41-50, >50 years) as well as their geographic localization.

The results show significant differences when a comparison is performed in function of gender and geographic localization. These results emphasize the need to check the S levels in Brazilian diet and they can also be useful in other research areas such as health and nutrition.

2. Supplementation

1-4

IRON ACCUMULATION IN TISSUES OF RATS ADMINISTERED A LARGE AMOUNT OF FERRIC CITRATE

Munehiro YOSHIDA*, Hirohisa YAMAKAWA, Kenji FUKUNAGA

*Laboratory of Food and Nutritional Sciences, Faculty of Chemistry, Materials and Bioengineering, Kansai University, Suita Osaka, 564-8680, Japan.*E-mail: hanmyou4@kansai-u.ac.jp*

In patients with a renal failure, control of the absorption of dietary phosphate is necessary to prevent hyperphosphataemia. Therefore, most patients are treated with orally administered aluminum, calcium or lanthanum salts that bind dietary phosphate and facilitate fecal elimination rather than intestinal absorption. Recently, it has been proposed that ferric citrate is used as a phosphate-binder for patients with renal failure. However, some recent studies have indicated that an accumulation of iron in liver causes cirrhosis and liver cancer. In the present study, we examined iron accumulation in tissues of rats administered a large amount of ferric citrate.

Male 4-week-old Wistar rats were fed AIN93G diet (basal diet) or the basal diet supplemented with 0.25 or 1.0% iron as ferric citrate for 4 weeks. Administration of 1.0% iron significantly inhibited the rat growth. However, liver function and lipid metabolism were not influenced by the iron administration. Serum phosphate was significantly lower in the 1% iron group compared to the other two groups. Serum iron and transferrin saturation (Tf-saturation) was dose-dependently increased by the iron administration. In particular, Tf saturation of the 1.0% iron group was near 90%. Iron in the liver, kidney, spleen and tibia were dose-dependently increased by the iron administration. In particular, iron in liver of rats fed the 0.25 or 1.0% iron diet was 3 or 8 times higher than that of rats fed the basal diet, respectively. Zinc, copper and manganese contents in the liver, kidney, spleen and tibia were not clearly influenced by the iron administration. These results indicate that oral administration of a large amount of ferric citrate causes a saturation of transferrin and a high iron accumulation in liver.

THE IMPACT OF SELENIUM SUPPLEMENTATION AND GENETIC POLYMORPHISM OF SELENOPROTEINS ON HBA1C AND BLOOD LIPID PROFILE - PRELIMINARY RESULTS OF THE SUPPLEMENTATION TRIAL CONDUCTED IN THE POLLISH INDIVIDUALS

Jablonska E1., Krol M1., Gromadzinska J1., Reszka Et., Wieczorek E1., Socha K2., Borawska MH2., Wasowicz W1.

1 - Nofer Institute of Occupational Medicine, Lodz, Poland, e-mail: wojciech@imp.lodz.pf
2 - Medical University of Bialystok, Poland, e-mail:borawska@umb.edu.pl

Selenium (Se) is an essential trace element required for proper functioning and development of human organism and it is supposed to be involved in the prevention of chronic diseases such as cancer, cardiovascular disease and diabetes. However, recent findings raise serlous concern about the association between high selenium status and diabetes or hyperlipidemia. Interestingly, recent nutrigenetic etudies on selenium suggest that the biological effect of selenium supplementation may depend on genotype related to polymorphic variants in selenoprotein encoding genes. Based on this hypothesis, an intervention study was deslgned in order to assess the influence of the genetic polymorphism of certain selenoproteins on the markers of diabetes and dyslipidemia in the indlviduals supplemented with selenium. The supplemented group included 95 individuals aged 18-60 (43 men and 52 women) who were selected for the study according to the possessed genotype, associated with two functional SNPs located in two selenoprotein encoding genes (cytosolic glutathione peroxidase and selenoprotein P). All individuals received 200 µg of selenium in the form of selenium yeast, for six weeks. During the supplementation trial, blood for analysis was collected at four time points: before the supplementation, after two and six weeks of supplementation and after four weeks of washout period. The analysis comprised plasma selenium concentration, blood HbA1c (glycated hemoglobin; marker of prediabetes or diabetes) and markers of blood lipid profile: total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides. Preliminary results of the study, indicating the impact of genotype and Se supplementation on the analyzed biomarkers in the context of the risk of diabetes and cardio-metabolic disorders, will be presented.

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DIETARY BORON DOES NOT AFFECT TEETH STRENGTH, MICRO-HARDNESS, AND DENSITY, BUT AFFECTS MINERAL COMPOSITION AND ALVEOLAR BONE MINERAL DENSITY IN RABBITS FED A HIGH-ENERGY DIET

Hakki SS[1], Malkoc S[2], Dundar N[3], Kayis SA[4], Hakki EE[5], Hamurcu M[5], Baspinar N[6], Basoglu A[7], Nielsen FH[8], Götz W[9].

1 *Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya 42079, Turkey, sshakki@yahoo.com*
2 *Inonu University, Faculty of Dentistry, Department of Orthodontics, Malatya, Turkey*
3 *Selcuk University, Research Center of Faculty of Dentistry, Konya, 7 Turkey*
4 *Selcuk University, Faculty of Agriculture, Department of Animal Science, Konya, 8 Turkey*
5 *Selcuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Konya, 9 Turkey*
6 *Selcuk University, Faculty of Veterinary Medicine Department of Biochemistry, Konya, 11 Turkey*
7 *Selcuk University, Faculty of Veterinary Medicine, Department of Internal Medicine, Konya, 12 Turkey*
8 *University of North Dakota, Grand Forks, North Dakota, 58202, USA*
9 *Bonn University, Medical Faculty, Department of Orthodontics, Oral Biology Lab, Bonn, Germany*

The objective of this study was to determine whether dietary boron (B) affects the strength, density and mineral composition of teeth and alveolar bone density in rabbits with apparent obesity induced by a high-energy diet. Sixty female, 8-month-old New Zealand rabbits, were randomly assigned for 7 months into five groups as follows: 1) control 1, fed alfalfa hay only (5.91MJ/kg); 2) control 2, high energy diet (11.76MJ and 3.88 mg boron/kg); 3) B10, high energy diet+10mg/kg body weight boron gavage/96h; 4) B30, high energy diet+30mg/kg body weight boron gavage/96h; 5) B50, high energy diet+50mg/kg body weight boron gavage/96h. Maxillary incisor teeth of the rabbits were evaluated for compression strength, mineral composition and micro-hardness. Enamel, dentin, cementum and pulp tissue were examined histologically. Mineral densities of the incisor teeth and surrounding alveolar bone were determined by using micro-CT. When compared to controls, the different boron treatments did not significantly affect compression strength, and micro-hardness of the teeth, although the B content of teeth increased in a dose-dependent manner. Phosphorus (P), sulfur (S), and zinc (Zn) content decreased with the increasing B level of diet. Histological examination revealed that teeth structure (shape and thickness of the enamel, dentin, cementum and pulp) was similar in the B-treated and control rabbits. Micro CT evaluation revealed that while increased alveolar bone mineral density was noted in B10 and B30 groups when compared to controls, alveolar bone density was not positively affected in B50 group. Although B has no further effects on the structure, strength, mineral density and micro-hardness of the teeth, dietary B regulates composition of other minerals in the teeth and it has beneficial effects on the mineral density of the surrounding alveolar bone.

1-7

HEPATOPROTECTIVE EFFECT OF SELENIUM AND ITS ORGANIC DERIVATIVES OF TOXIC HEPATITIS

K.O. Sharipov, Doctor of Biochemistry Sciences *skamalidin@mail.ru*

Kazakh National Medical University - Names after C.D. Kazalhstan, Almaty

Selenium is essential trace element which plays an important role in protecting the organism from the toxic effect of reactive oxygen species and lipoperoxides. That explains the antioxidant activity in protective effects of selenium deficiency of vitamin E, as well as the effects of xenobiotics and other factors contribute to the process of free radical oxidation (FRO) of the lipids.

In the present work are results of the study of the influence of new compounds of selenium on intensity of lipid peroxidation (LPO) and the activity of antioxidant enzymes and antioxidant activity of the liver with acute phosphorus intoxication (AFI), and compare them with the therapeutic effect of sodium selenite.

The research was conducted on inbred albino male rats. Toxic hepatitis of phosphate genesis reproduced through the intragastric injection of a water suspension of yellow phosphorus.

Selenium piperidine (dialkyl) selenophosphat (PSP) was synthesized in the Institute of Chemistry of the National Academy of Sciences of the Republic of Kazakhstan. Their structure was proved by infrared (IR) spectroscopy, proton magnetic resonance (PMR) and the composition established by a method of elemental analysis. Determination of acute toxicity (LD50) was carried out on white mice.

The acuter phosphoric intoxication is intensifies processes of FRO of lipids, activation parameters of chemiluminescence (CL), and depression of antioxidant system in rat liver.

The injection of selenium against API increases the survival of the animals, reducing CL indicators, normalization of lipid peroxidation and induction of antioxidant-enzymes: superoxide dismutase, glyutatinonperoxidase, glutathione reductase and catalase. At the same PSP shows significantly superior AO effect than sodium selenite.

The dominant effect of AO-PSP compared to sodium selenite, apparently due, first, to the ability of the cationic atom of nitrogen pick reactive oxygen species (O_2^-, O., .OH) and phosphorus (R., RO., ROO.) with following their utilization to normal metabolism, and secondly to increase effect the AO-protection of cells effect of elemental selenium.

Conclusions: Organic selenium PSP, which containing in the structure of cationic atom of nitrogen shows antioxidant and hepatoprotective effects significantly ($P < 0.05$) superior to sodium selenite, in this case, PSP is less toxic. In the present time organic selenium PSP will be clinical trials.

ELECTROMAGNETIC TECHNOLOGIES IN BIOELEMENTS PRODUCTION: THE DEVELOPMENT OF BIOELEMENTOLOGY

Tambiev A. Kh. [1], Skalny A.V. [2,3]

[1] *M.V.Lomonosov Moscow State University, Moscow, Russia*
[2] *Trace Element – Institute for UNESCO, Lyon, France*
[3] *Russian Society of Trace Elements in Medicine, Moscow, Russia*
E-mail: skalny3@microelements.ru

According to conception of bioelements and bioelementology as a new integrative approach in life sciences, proposed by A.V.Skalny (2003-2011) the existing of biosystems depends on combination of internal factors (presence of bioelements as blocks of life) and external conditions including electromagnetic fields. Biosphere is an assembly of bioelements and living organisms existing under permanent regulatory influence of physico-chemical factors of terrestrial and cosmic origin. We have first (Tambiev et al., 1997) identified a significant biological effect of these waves on the photosynthetic organisms: cyanobacteria and microalgae. It was shown in many studies that water retains the information about the history of physical influences on it, which significantly affects the flow of processes in the aquatic environment and creates opportunities for developing new channels of control of chemical, biochemical and biological processes. The experiments showed that millimeter waves can influence on the chemical (mineral) content of cells of the photosynthetic organisms – cyanobacteria and microalgae, which are widespread objects of photobiotehnology. We have managed to significantly increase the synthesis of so-called secondary bioelements by microalgae Spirulina platensis and Spirulina maxima. Under the influence of millimeter wave radiation there is observed increased accumulation of a number of trace elements from the environment: selenium, chromium, zinc, copper, lithium, etc., with dramatic change in elemental composition of the algal cells (Tambiev et al., 2000-2011). Our studies indicate the applicability of electromagnetic millimeter waves for the efficient biosynthesis of secondary bioelements and the necessary building blocks for life maintenance, and finally for increasing the mass and diversity of living matter on the planet, which has an undoubted theoretical and practical importance.

SELECTION ALGORITHM CONSTITUTIONAL MEDICINE MINERAL SERIES IN HOMEOPATHY

Palcu Akaeva T.V.[1], Mkhitaryan K.N.[2]

1 *I.M. Sechenov First State Moscow Medical University RF Ministry of Health Development, Laboratory for Development and Implementation of Novel Drug-Free Therapeutic Methods, Moscow, Russia*
2 *Limited Liability Company Centre of Intellectual Medical Systems «IMEDIS», Russia*
akaevatatiana@gmail.com

Introduction. Currently, specialists engaged in traditional homeopathy are unaware of how and by which rigorous criterion it is possible to distinguish between the constitutional and non-constitutional homeopathic (or other, informational) preparations, as well as how at all such a criterion should be formed. The present study was aimed at comparing the efficacy of choosing a constitutional homeopathic agent from the group of mineral series (impairments of element metabolism) and various methods of drug-free therapy with electronic autonosode of blood of the patient.

Aims of the study:

To compare the results of therapy for impaired element metabolism with autonosode of the patient's blood, depending on the targeting marker employed.

To determine and duly assess the tentative prognosis of residual impairments of elements and the possibilities of determining the constitutional agent from the group of impairments of element metabolism.

Objectives:

Using compensation of impaired element metabolism, by the example of potentiated elements, to formulate a criterion of constitutionality of a homeopathic agent which would simultaneously be as follows:

therapeutically significant (meaningful) – so that the drugs selected with the help of it would provide effective therapy of the patient.

constructive, i.e., allowing exact wording and checking up within the framework of the diagnostic methodology of the vegetative resonance testing (VRT).

Patients and methods: In order to make diagnosis by means of VRT and to produce the patient's blood autonosodes, we used the hard/software complex (HSC) for electropuncture diagnosis, medicamentous testing, adaptive bioresonance therapy, as well as electro-, magnetic and light therapy on the biologically active points (BAPs) and biologically active zones (BAZs) «IMEDIS-EXPERT». The study was carried out on a sample comprising a total of seventy-two 18-to-65-year-old patients presenting with various chronic nosological entities. All patients underwent primary VRT diagnosis according to the common algorithm in accordance with the approved methodology. Impairments of element metabolism were determined by 17 positions corresponding to the test indices of element metabolism in the VRT test. The patients were randomly

divided into two groups, each consisting of 36 people. Group One patients received therapy with blood autonosode whose potency was chosen so that to compensate the so-called systemic KMH* marker, by means of turning the potency regulator we performed potentiation of blood autonosode, up to meeting the VRT conditions – compensation of autonosode-potentiated load with the KMH marker and achieving reproducibility of the results of measuring (reproducible elevation of the "measuring needle" up to 100 conventional units on the TI):

KMH ↓ +Potα(AHKp)↑ (1)

The KMKh marker is the sum of electron signals from the end points and the points of intersection of the main chiroglyphic lines located on the palmar surface of the patient's hand. Group Two patients received therapy with potentiated blood autonosode whose potency was chosen so that to compensate the sum marker of impairments of element metabolism (SMIEM):

SMIEM↓ +Potβ(AHKp)↑ (2)

Then during two months, we treated our patients from group 1 and 2 with blood autonosode compensating the markers of KMH and SMIEM, for patients from group 1 and 2, respectively. During the secondary VRT testing we performed checking of the test indices of impaired element metabolism but with filtration through pseudotransparent KMKh markers and SMIEM in the respective groups using the following schemes of testing:

KMH ↓+Potα(AHKp)↑+Element↕ (3)

for the first group, and for the second group

SMIEM↓+Potα(AHKp)↑+Element↕ (4)

The prognosis of the residual impairments of element metabolism was made by means of various regimens of testing using the scheme (3) in the first and (4) in the second groups, with the therapy in them performed using the same blood autonosode which was also employed for making the prognosis. In order to assess the prognostic significance in relation to determining the constitutional agents we used the following:

number (%) of patients with inclusion or coincidence (criterion of constitutionality) of the set of the test indices of the prognosis of residual impairments of element metabolism, revealed at the first visit by the VRT testing with the set of test-indices of constitutional impairments of element metabolism, detected by means of testing during the secondary examination of patients. The obtained results were processed using the statistical Fisher criterion φ* and its modifications.

Results. The obtained findings have led the authors to understanding and using of a strict definition of the notion of constitutional homeopathic preparation within the frameworks of the methodology of combined use

* An acronym from the surnames of the authors: Kudaev-Mkhitaryan-Hodareva [interpreter's note]

of VRT-BRT. Proceeding from the homeopathic practice under the VRT control and using the KMH marker, we propose a formal VRT criterion of constitutionality (constitutional orientation) of the agent in the form of the VRT condition:

KMH↓+Potα(Agent)↑ (5)

with the condition (5) supposed to be complied with irrespective of the choice of the potency α. The sense of this definition consists in the fact that the action of the constitutional agent is considered as compensating summed signal of BAT-"models birth-death" and BAT-"models of the main conflicts of self-fulfilment" presumably located, respectively, in the terminal and nodal points of the main chiroglyphic lines of the patient's palms.

Using the pseudotransparent marker KMH+PotαAHKp for prognosis of impaired element metabolism, the constitutional element chosen by criterion (5) coincided with the residual one in 95% of cases, i.e., the most persistent impairment of element metabolism after two months of therapy. The proposed criterion seems to have practical significance: its use for selection of homeopathic drugs yields a good therapeutic effect. The findings of our study show that if limiting as constitutional agents by potentiated elements only, the notion of the constitutional homeopathic agent is objective – this is the most persistent impairment of element metabolism which remains after therapy with blood nosodes (general systemic therapy) and which statistically significantly ($p \leq 0.01$) compensates the systemic marker KMH.

Conclusions:

The carried out study using the VRT method made it possible to confirm the tentative prognosis of residual impairments of elements by the example of therapy for elementoses with the use of the KMH and SMIEM markers. It was shown that the prognostic significance using the KMH marker varied form 62.9 to 91.36% ($p \leq 0.01$), with the prognostic significance of using the systemic KMH marker being reliably higher than that using the SMIEM marker ($p \leq 0.01$).

The findings obtained in the present study have made it possible to substantiate the previously existing assumptions concerning objectivity of the notion of the constitutional homeopathic agent as applied to potentiated elements, as well as to offer a clinical model of therapy within the frameworks of the diagnostic methodology of VRT, allowing of revealing the constitutional element.

INTERVENTION OF A-LIPOIC ACID ALONG WITH NUTRITIONAL SUPPLEMENT AMELIORATES LEAD INDUCED OXIDATIVE STRESS AND DNA DAMAGE

Sangeeta Shukla, Samta Sharma and Yamini Sharma

Reproductive Biology and Toxicology Laboratory
School of Studies in Zoology,
Jiwaji University, Gwalior 474011, MP India.
Email: profsshukla@gmail.com

Environmental contamination and exposure to heavy metals contribute to serious health hazards. Metals such as mercury, cadmium and lead are a serious growing problem throughout the world. Among all metals lead is one of the sixth most serious pollution threats to the planet. The quantity of lead used in the 21th century far exceeds the total consumed in all previous eras, thus scientific attention is focused because of its potential health risks. With increasing concerns about environmental pollution, the interaction of micronutrients and antioxidants with toxic metals is of great interest. The assumption of oxidative stress as a mechanism in lead toxicity suggests that antioxidants might play a role in the treatment of lead poisoning. We investigated the effects of LA alone or in combination with zinc in influencing the lead induced alterations in haem synthesis pathway, hepatic, renal and brain oxidative stress and lead concentration from blood and soft tissues.The present study was designed to investigate the efficacy of lipoic acid (LA) along with zinc in rebalancing the increased prooxidant/antioxidant ratio in lead-exposed rats. Male Wistar rats weighing 150±10 g were exposed to lead intraperitoneally at the dose of 50 mg/kg body weight for three day along with lipoic acid and zinc at the dose of 100 mg/kg and 10 mg/kg respectively. Lead exposure resulted in alteration in haem synthetic enzymes ALAD, ALAS. A significant increase in MDA levels, serum transaminases, urea, uric acid creatinine triglycerides and cholesterol while substantial decrease in GSH content and AChE enzyme activity was observed. Also the concentration of lead in blood and soft tissues was elevated. Both the thiol chelator and nutritional supplement were able to increase blood ALAD activity and GSH level towards normal. All the treatments were able to recoup altered biochemical variables towards normal The most prominent beneficial effects, however, were observed in animals treated concomitantly with LA and zinc. The results thus provide evidence of an encouraging role of LA when given in combination with zinc in the therapeutic intervention of lead poisoning, particularly in reducing the oxidative stress and lead concentration.

3. Measurement

SERUM REFERENCE RANGES FOR TITANIUM AND OTHER ULTRA-TRACE ELEMENTS

Barry Sampson[1], Alan Courtney[1], Therese Panetta[1], Oliver Pearce[2] & Alister Hart[3]

1 Clinical Biochemistry, Charing Cross Hospital and Imperial College School of Medicine, London W6 8RF, UK - b.sampson@imperial.ac.uk
2 Oliver Pearce, Orthopaedic Surgery, Milton Keynes Hospital, MK6 5LD, UK
3 Alister Hart, Institute of Orthopaedics, Royal National Orthopaedic Hospital, Stanmore, HA 4LP, UK

Background Measurement of blood metal ions is an important part of the evaluation of patients with metal implants, especially patients with metal-on-metal hip replacements. These have recently been subject to regulatory advice in several countries. Some implants have been withdrawn by the manufacturers. The weight bearing surfaces of these implants are made from a Co-Cr-Mo alloy, but other metals are used in some components. Material loss from the head-stem junction is considered an explanation for the high rates of adverse tissue reactions seen with large head metal-on-metal; modular neck; and large head metal-on-polyethylene hip replacements. The stem is commonly made of Ti and in such cases blood levels of up to 85 nmol/L (4ug/L) have been measured. However, the levels are not yet known in patients without Ti-containing implants.

Methods: Blood samples were collected from 22 volunteers using trace element blood collection tubes. Trace element concentrations were measured in high resolution ICPMS. Blank tubes were also assessed for contamination.

Results Tube blanks: Ti <15 nmol/L; Cr <2 nmol/L; Co, Mo, V <1 nmol/L; Ni < 5 nmol/L. Control serum samples: Ti 13.6±16.0 nmol/L (1 outlier, 122 nmol/L); Cr all samples below detection limit (DL) 5 nmol/L; Co 6.0±1.9 nmol/L (2 outliers, 25,46 nmol/L); Mo 11.4±4.6 nmol/L (1 outlier,43 nmol/L); V 1.6±0.8 nmol/L; Ni 14.8±7.0 nmol/L (1 outlier, 201 nmol/L).

Conclusions Trace element sample tubes are suitable for the collection of samples for measurement of ultra-trace elements. There is some Ti contamination, but will not have much clinical effect. The results for control sera agree with previously published data for Cr, Co, Mo, Ni and V. The availability of reference range data for Ti will allow interpretation of measurements in samples from patients with metal containing surgical implants.

1-12

INVESTIGATION OF INORGANIC ELEMENTS IN SALIVA FROM *AMBLYOMMA CAJANNENSE* SPECIES FROM BRAZIL BY THE EDXRF TECHNIQUE

Ivone. M Sato[1], Cibele B. Zamboni[1], Daniella G. L. Oliveira[2], Ana M. Chudzinski-Tavassi[2], Simone M. Simons[2]

1 Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN/SP
Av. Professor Lineu Prestes 224205508-000 São Paulo, SP, Brazil
imsato@ipen.br
czamboni@ipen.br
2 Instituto Butantan Laboratório de Bioquímica e Biofísica Av Vital Brasil 1500 –05503-900 São Paulo, SP – Brazil
daniellagloliveira@gmail.com /
amchudzinski@butantan.gov.br
simonemsimons@yahoo.com.br/

The Amablyomma Cajennense tick is one of the most widespread species in Brazil. This tick is subject of studies related to antihemostatic properties and antitumor agents as well as a vector of diseases. Recently, the NAA technique has been applied to its chemical characterization. Now, this investigation is intended to be continued using the EDXRF technique. The biological material was obtained from Butantan Institute (São Paulo city, Brazil). For the saliva samples, it was necessary to maintain a colony of ticks with 30 females and 15 males, during approximately 7 months. This work was developed in collaboration with the Laboratory of Parasitology at Institute Butantan, where the colony was kept in an incubator with controlled temperature, humidity and oxygenation conditions. The saliva was collected into microcapillary tubes attached to the female hypostome and transferred to filter paper (Whatman – No. 42) in aliquots of 50 µL and 100 µL. The reference values were determined for Ca, Cl, Cu, K, Mg, Na, P, S and Zn and a comparative study of EDXRF and NAA results were carried out. These data contribute to the understanding of saliva composition, complementing its characterization as well as for the understanding of the many physiological processes related to salivary secretion. This study, also, adds expertise in several research areas, such as the formulation of vaccines and other therapeutic targets.

NOVEL MATHEMATICAL MODEL FOR CALCULATION OF STOICHIOMETRY OF IRON COMPLEXES

Tomáš Filipský1, Michal Říha1, Radomír Hrdina1, Kateřina Vávrová2 and Přemysl Mladěnka1

Charles University in Prague, Faculty of Pharmacy in Hradec Králové
1Department of Pharmacology and Toxicology,
2Department of Inorganic and Organic Chemistry,
Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
Email: tomas.filipsky@faf.cuni.cz

Iron is a crucial element of all human cells. Its ability to easily convert between two oxidation states Fe(III)/Fe(II) under physiological conditions makes it an essential catalytic component in many biochemical reactions. Contrarily, iron is also able to promote the production of toxic oxygen radicals (Fenton reaction), particularly when it is present in excess. Iron chelators may inhibit/facilitate this reaction depending on their affinity to Fe in relation to pH.

Since various pathologies disrupt normal pH and lower complex stoichiometry can particularly be associated with weakly bound iron with consequent catalysis of the Fenton reaction, this study was aimed at the analysis of iron complexes at different relevant pHs.

In our study, a new complementary approach for the assessment of stoichiometry was evolved. The complexes of 10 different known iron chelators (deferoxamine, deferasirox, deferiprone, EDTA, 8-hydroxyquinoline, chloroxine, SIH, PIH, quercetin and rutin) were analyzed by the direct UV-Vis spectrophotometry at different pHs (4.5, 5.5, 6.8 and 7.5). The standard Job's method was used for the comparison.

This study showed that the simple approach based on absorbance at the wavelength of complex absorption maximum was sufficient when the difference between absorption maximum of substance and complex was high. However, in majority of substances this difference was much lower. Particularly, the theoretical absorption lines mimicking the absorption of the tested compounds were more suitable for the analysis. The major benefit of this method compared to the standard Job's approach seems to be its capability to reveal a reaction stoichiometry in chelators with moderate affinity to iron.

In conclusion, using this complementary method may explain several previous contradictory data and lead to a better understanding of the underlying mechanisms of chelator's action.

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ANALYSIS OF THE ELEMENTAL AND CHEMICAL COMPOSITION OF HUMAN BIOLOGICAL MATERIALS BY APPLICATION OF X-RAY FLUORESCENCE AND X-RAY DIFFRACTION METHODS

W. Kurtek 1, K. Bielecka 1, D. Banas 1,2, J. Braziewicz 1,2, A. Kubala-Kukus 1,2, U. Majewska 1,2, M. Pajek 1, J. Wudarczyk-Mocko 2

1 Institute of Physics, Jan Kochanowski University, 25-406 Kielce, Poland
*2 Holycross Cancer Center, 25-734 Kielce, Poland *d.banas@ujk.edu.pl*

The x-ray fluorescence and diffraction techniques offer unique possibilities for analysis of the elemental and chemical composition in various types of samples. These techniques are particularly weil suited for biomedical and environmental sam pies to investigate the dynamics of elements transport, accumulation processes and, generally, the influence of environ mental pollution on a level of trace elements and compounds in human organism. This can be achieved by correlating the trace elements and compounds content in medical samples, such as, for example: bones, teeth, solid concretion or crystal aggregation in human body, with the same content measured in environmental samples (food, air, water, soil, plants).

The main aim of presented studies is analysis of the elemental and chemical composition of human biological samples such as bones, teeth, kidney stones, gallstones and urinary stones by application of total reflection x-ray fluorescence (TXRF), wavelength dispersive x- ray fluorescence (WDXRF) and x-ray powder diffraction (XRPD) methods. The performed WDXRF and TXRF studies allowed on the analysis of the medical sam pies in wide range of elements (from oxygen to lead) and broad values range of the determined concentration (from tens of ppb to tens of percent). On the other hand the XRPD method gave information about the chemical composition of the analysed samples. In this work the experimental systems, methodology of samples preparation and method of carrying out the measurement will be described. The results will be interpreted in the context of possible environ mental factors that can affect the obtained results of elements and compounds content.

1-15

AN ALYSIS OF ELEMENTS CONCENTRATION IN TOBACCO PLANT LEAF, PIPE AND SMOKER BIOMEDICAL SAMPLES BY APPLICAT ION OF TXRF METHOD

U. Majewska1 , 2 *, I. Sychowska1 , M. Soltysiak1 , D. Banas1 , 2 , A. Kubala-Kukus1 , 2 , J. Wudarczyk-Mocko2 , J. Braziewicz1 , 2 ,M. Pajek1

1) Institute of Physics, Jan Kochanowski University, 25-406 Kielce, Poland
2) Holycross Cancer Center, 25-734 Kielce, Poland
**Urszula.Majewska@ujk.kielce.pl*

The contents of trace elements in biological and biomedical samples are of great interest for biology, medicine and environmental sciences. In the studies of concentration of trace elements using different analytical techniques, one of the most known is the total reflection X-ray fluorescence (TXRF) method, which is very well suited particularly in the biological and medical samples. This results from the fact that the organic samples are built mainly of light elements, which facilitates an observation of heavy element contamination using X-ray spectrometry. Additionally, the organic materials can be easily dissolved and mixed with an internal standard, which allows on fast and accurate (~15%) analysis of all trace elements heavier than silicon (Z > 14). For this reason the TXRF method is routinely used in biological, medical and environmental applications. The main aim of presented studies is analysis of elements concentration in tobacco plant leaf, pipe and smoker biomedical samples and study of correlation between elements concentration in tobacco plant leaf and pipe and their contents in biomedical human samples (for example urine). Work presents the values of analysed elements concentrations in samples mentioned above, detection limit value for each kind of samples and short description of experimental set-up, sample preparation procedure and calibration procedure for each kind of samples.

1-16

DETERMINATION OF REFERENCE VALUES OF TRACE ELEMENTS CONCENTRATIONS IN HUMAN SERUM, URINE AND HAIR SAMPLES

A. Kubala-Kuku 1, 2 , * , D. Bana 1 , 2 , J. Braziewicz1 , 2 , U. Majewska1 , 2 , M. Pajek1 , I. Sychowska1, J. Wudarczyk-Mo ko2 , G. Antczak2 , B. Borkowska2 , S. Gó d 2

1 Institute of Physics, Jan Kochanowski University, 25-406 Kielce, Poland
2 Holycross Cancer Center, 25-734 Kielce, Poland
** a.kubala-kukus@ujk.edu.pl*

Knowledge of the reference values of trace element concentrations in the human body is often very important for a proper assessment of organism condition. Due to the large variety of the substances concentration in body fluids and tissues inside the human population, determination of the reliable reference values requires adequate size and selection of the control group. In addition, it is necessary to develop clear procedures for sampling and sample analysis and controlling of stability of measuring apparatus. Very important for the final reference limits is also a statistical analysis of the results taking into account the sensitivity of the analysis methods used.

The aim of this study was obtaining reference values of concentration of trace elements in human serum, urine and hair. For the sample analysis, well known total reflection x-ray fluorescence (TXRF) method was used. This method allows to determine the concentration of trace elements heavier than aluminum with a detection limit on 10 ng/g level. We will describe the method of selection of the control group, the experimental system and its calibration procedure, method of samples preparation for analysis and, finally, the results which were obtained. Additionally, statistical analysis of the results and determined reference values of concentration of trace elements in human serum, urine and hair will be presented.

1-17

BATHOCUPROINE METHOD FOR THE COMPARISON OF COPPER CHELATION AT (PATHO)PHYSIOLOGICALLY RELEVANT PH CONDITIONS

Michal Riha1, Jana Karlfëkovàz, Tomas Filipskyt, Katefina Macàkovàz, Radomir Hrdina1 & Premysl Mladënka1

Charles University in Prague, Faculty of Pharmacy in Hradec Kràlové, Heyrovského 1203, 50005 Hradec Kràlové, Czech Republic
1 Department of Pharmacology and Toxicology
2 Department of Pharmaceutical Botany and Ecology
E-mail: riham6aa@faf.cuni.cz

Trace metal copper plays an indispensable role in the human body, being involved in many physiological processes. However, disorder of copper homeostasis at systemic or local levels can be observed in various pathologies, thus, copper chelation may represent a promising therapeutic tool.

The aim of this study was to: 1) create an in vitro methodology for screening of copper chelators, and 2) analyse in detail the interaction of copper with clinically used D penicillamine (D-PEN) and trientine, experimentally tested 8-hydroxyquinolines; and EDTA for comparison.

Spectrophotometric methodology based on bathocuproinedisulphonic acid disodium salt (SCS) enabled both cuprous and cupric ions chelation assessment at (patho)physiologically relevant pHs (4.5-7.5). Potent chelators EDTA, clioquinol and chloroxine formed stable complexes with both cupric and cuprous ions at ail selected pHs. Copper complexes of EDT A had 1: 1 stoichiometry while those of clioquinol and chloroxine 2:1. Trientine formed stable complexes of 1:1 stoichiometry at neutral pH but its affinity dropped with decreasing pH. Non-substituted 8-hydroxyquinoline was clearly less active while, unexpectedly, the affinity of D-PEN for copper was the lowest from the tested substance. Moreover, D-PEN was the only chelator which was able to reduce cupric ions.

Concluslvely, SCS assay represents a rapid, simple and precise method for copper chelation measurement. In addition, lower binding affinity of D-PEN compared to trientine and 8-hydroxyquinolines may have a clinical relevance.

The work was supported by Charles University (grants No. 605712C and 265 003).

COMPARATIVE STUDY OF INORGANIC ELEMENTS DETERMINATION IN WHOLE BLOOD FROM DMDMDX/J MICE STRAIN BY EDXRF AND NAA ANALYTICAL TECHNIQUES

M. M. Redigolo [1], I. M. Sato [1], S. Metairon [2] and C. B. Zamboni [2]

1 *Instituto de Pesquisas Energéticas e Nucleares - Centro de Química e Meio Ambiente*
Av. Prof. Lineu Prestes, 2242 – Cidade Universitária - 05508-000 São Paulo, SP Brazil
2 *Instituto de Pesquisas Energéticas e Nucleares – Centro de Reatores de Pesquisa*
Av. Prof. Lineu Prestes, 2242 – Cidade Universitária - 05508-000 São Paulo, SP Brazil
marcelo.redigolo@usp.br, imsato@ipen.br

The Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disease that affects 1/3,500 boys. The symptoms begin at 5 and the life expectancy is not more than 20 years. This disease is still incurable. Its main characteristic is the degeneration of the membrane that encloses the muscular cell (sarcolemma), leading to muscular necrosis due to calcium accumulation. One mouse strain used in DMD studies is the Dmdmdx/J. The relevance of the present work is supported by recent studies which relate variations on the concentration of inorganic elements in bodily fluids (blood, serum, urine) to the presence of diseases (cognitive, cardiovascular and cancer). In this work, whole blood samples (100 µL) from Dmdmdx/J (9 males and 10 females) dystrophic mice and C57BL/6J (10 males) control group were analyzed by the EDXRF technique. Reference interval values were determined for Na, Mg, S, Cl, K, Ca, Fe and Cu. A comparative study with NAA results was carried out. Samples were obtained from renowned Brazilian research centers, such as IPEN, Instituto Butantan and Instituto de Biociências. Both techniques (EDXRF and NAA) showed to be appropriate for whole blood sample analyses and offer a new contribution for Veterinary Medicine blood analyses.

1-19

ARE ROBUST STATISTICS AND QUALITY SPECIFICATION BASED ON INDIVIDUAL VARIABILITY SUITABLE FOR SERUM AI PROFICIENCY TESTING

J Arnaud[1], I Coté[2], P Parsons[3], M Patriarca[4], A Taylor[5], C Weykamp[6]

1 *DBTP, pôle de biologie, CHU de Grenoble, F-38043 Grenoble Cedex 9*
2 *Centre de Toxicologie, Institut National de Santé Publique du Québec, 945 avenue Wolfe, Québec, G1V 5B3, QC Canada,*
3 *Laboratory of inorganic and nuclear chemistry, Wadsworth Center, New York State Department of Health PO Box 509, Albany, NY 12201-0509, USA,*
4 *Department of Public Veterinary Health and Food Safety, Istituto Superiore di Sanita, Viale Regina Elena 299,00161 Rome, Italy,*
5 *Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH, United Kingdom, 6 - MCA Laboratory, Queen Beatrix hospital, 7101 BN Winterswijk, The Netherlands*

All the authors are members of the thematic network "Organisers of external quality assessment / proficiency testing schemes related to occupational and environmental medicine"

Background: Trace element proficiency testing schemes monitor laboratory performance and provide a stimulus for improvement in accuracy. Monitoring of participant's performance depends on the methods used to determine the assigned values and the standard deviation for proficiency testing (SDPT).

Applied techniques: Samples (one native pool of human serum and two further serum pools prepared by spiking the first with known AI concentrations) were sent to all participants of the French, Italian, New York and United Kingdom proficiency testing schemes. Results were returned to the respective scheme organizers. Consensus robust means (algorithm A) and robust standard deviations were calculated. The robust consensus means were taken as the assigned values. Participant Z-scores were also calculated using SDPT derived from quality specifications, based on individual variability (1) previously defined by the network (2,3). Additional data analysis was carried out to evaluate the recovery of spikes and the performance of different analytical techniques.

Important results: A satisfactory recovery of spike (101 %) was observed. Although the ANOVA test remained not statically significant, the recovery was significantly lower using ICP/OES than EAAS. Numerous outlier results (Z-score <-3 or >3) were observed (14%) mainly in the upper values indicating that contamination is not under control in all the participant laboratories. In addition, 11 % of results were questionable. Finally, ICPMS provided better results (p=0.05) than the other methods. In view of the contamination issues associated with measurement of this element, consensus robust statistics and quality specification based on individual variability may not be fit for purpose.

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1-20

IODINE AND SELENIUM MAPPING IN ENDOCRINE ORGANS USING WAVELENGTH-DISPERSIVE SPECTROMETRY (WDS)

Nadezda L Basalaeva : MD, PhD, senior inspector of Regional Directorate for Medical Provision at South Ural Railways.

The Territorial Branch of Russian Railways State-Owned Joint-Stock Company, Ul. Tsvillinga 41, 454091 Chelyabinsk, Russia. Email: nadyabas@gmail.com

X-ray fluorescence (XRF) is a noninvasive method used to quantify microelements, but frequently employed energy dispersive x-ray analyzer is not sensitive enough to detect trace elements in extrathyroidal organs. The aims of this work were:

1. To construct iodine (I) and selenium (Se) maps in the thyroid, anterior pituitary, ovaries, testis, suprarenals, pancreas and hypothalamus by XRF (WDS) quantitative analysis similarly to mapping by EDS (were used 11 rats).

2. Using the similar technique to examine I and Se maps in the thyroid, anterior pituitary and suprarenals after thyroid blocking by potassium iodide (were used 27 female rats).

I and Se mapping in organs was estimated in terms of weight percent (wt%) of I-2 and Se-2 in dry tissue using WDS quantitative analysis. Points with detected I or Se were defined as positive points (IPP or SePP). The percentage of IPP or SePP was analyzed and calculated to the whole amount of points examined.

I and Se mapping by WDS demonstrated that the level of I and Se in PPs of endocrine organs is identical, apart from thyroid and testis. This suggests, that the level of I and Se in PPs in the majority of endocrine organs can be defined as a "basic level". In organs with percentage of IPPs less than 50%, the percentage of SePPs augmented to 80% (testis, pancreas).

I content in endocrine organs changes both as I level in IPPs and as the percentage of IPPs; Se content – only as the percentage of SePPs.

I and Se mapping after thyroid blocking showed that I level in the thyroid IPPs (TIPPs) decreased gradually to "basic level", then normalized abruptly (rebound – effect). The gradual decrease of I level in the TIPPs was accompanied by the reduction of thyrotrophic hormone (TSH) expression in pituitaries (immunohistochemical method); maximal decrease of I level in the TIPPs accompanied abrupt growth of TSHs expression in pituitaries. Brief decrease of Se in SePPs of anterior pituitary and suprarenals preceded this effect.

CLINICAL APPLICATION OF TXRF SPECTROSCOPY

Armin Gross[1], Lena Telgmann[2] and Andreas Meyer[3]

1 Bruker Nano GmbH, Berlin, Germany, armin.gross@bruker-nano.de
2 Institute for Inorganic and Analytical Chemistry, University of Münster, Germany
3 Institute for Pharmaceutical Chemistry, University of Braunschweig, Germany

Analysis of gadolinium based MRI contrast agents

For more than 30 years magnetic resonance imaging (MRI) has been applied in medical diagnostics, providing non-invasive tomograms of the body. For image enhancement contrast agents which contain paramagnetic gadolinium (Gd^{3+}) ions are used. Due to its toxicity Gd^{3+} is complexed with chelating agents. Nonetheless, this contrast agents still cause severe kidney problems in case of certain diseases and have to be examined continuously.

Although numerous methods for the analysis of Gd-based contrast agents have been described, none of these methods are suitable for clinical routine analysis due to the complexity of sample preparation including digestion and dilution steps. In this paper the analysis of Gd in clinical samples by TXRF is described and data were compared with results from ICP-MS.

The concentrations of Gd in blood serum and urine determined by TXRF showed an excellent concordance with ICP-MS measurements. The samples of patients could be examined for their Gd excretion in less than half an hour after MRI examination.

Quantification of gold and platinum metallodrugs : The objective of this pilot study was the applicability of TXRF for the quantification of low concentrations in the µg/L range of gold and platinum based drugs in biological matrices (cell suspensions). For this purpose cisplatin, a common chemotherapeutic, and auranofin, a gold complex classified as antirheumatic agent, were chosen as relevant metal based drugs.

This paper shows that gold and platinum from metal based drugs can be quantified by TXRF in the ppb range with acceptable precision in aqueous samples as well as in cell suspensions. The easy preparation and handling of samples make TXRF very attractive and recommend it as an alternative method for clinical use. TXRF does not require any media or consumables and supports a cost-efficient laboratory practice.

GENDER DEPENDENT DIFFERENCES IN HAIR CALCIUM, POTASSIUM, MAGNESIUM, SODIUM, PHOSPHORUS AND ZINC

1Momčilović B, 2Prejac J, 1Višnjević V, 3Skalnaya MG, 4Drmić S, 5Mimica N,1Brundić S, 3Skalny AV.

1 Institute for Research and Development of the Sustainable Eco Systems (IRDSEC) Srebrnjak 59, 10 000 Zagreb (Zg), CROATIA (CR),
2 University Hospital Centre Zagreb, Kišpatićeva 12, Zg· CR,
3 Center for Biotic Medicine (CBM), Zemlyanoi val 46-48, 103604 Moscow, RUSSIA,
4 Neuropsychiatric Hospital "Dr. Ivan Barbot", Jelengradska 1, 44317 Popovača, CR,
5 University Psychiatric Hospital Vrapće, Bolnička cesta 32, Zg·CR.

Background. Hair (H) is a biomineralized composite tissue of high calcium content. In this study we analyzed the gender dependent frequency distribution of the hair Ca, K, Mg, Na, P, and Zn, as the role of gender in the intermediary metabolism and body status of these elements is poorly understood.

Methods. This prospective, observational, cross-sectional, exploratory, and epidemiological study involved 870 healthy Croatians (270 ♂ and 600 ♀). The hair calcium (CaH), magnesium (MgH), and sodium (NaH) were analyzed simultaneously with the ICP AES and hair potassium (KH), phosphorus (PH), and zinc (ZnH) with the ICP MS. The data were analyzed with the logistic sigmoid saturation curve of the median derivatives.

Results. The gross difference was observed for both the CaH and MgH intermediary metabolism; both of the elements were greatly higher in women than men. There were no gender dependent difference for the PH and ZnH intermediary metabolism between men and women. The liner portion of the saturation range of the logistic sigmoid curve was (μg·g-1): CaH (♂290 – 1200, ♀ - 4400), MgH (♂20 – 150, ♀ 40 – 450), NaH (♂ 90 – 1600, ♀ 60 - 1400), KH (♂ 40 – 1100, ♀ 20 - 500), and PH (120 – 200) and ZnH (130 – 260) for both men and women, respectively.

Conclusion. Gender is an important factor in the intermediary metabolism of CaH, MgH, KH, NaH, PH, and ZnH; every analyzed element showed a specific logistic sigmoid saturation curve of median derivatives. Regardless of the element, hair concentrations below the 20% saturation indicate an obviously inadequate intake, whereas those over the 80% indicate an overexposure. The dose-response range between the 20 and 80% was both proportional and highly linear for all the studied hair elements. We propose that the gender element specific logistic sigmoid curve of median derivatives should be saturated 60-80% if a plentiful dietary intake of Ca, Mg, Na, K, P, and Zn is preferred.

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HAIR IODINE FOR THE LONG TERM ASSESSMENT OF THE HUMAN IODINE STATUS

[1]Momčilović B, [2]Prejac J, [1]Višnjević V, [3]Skalnaya MG, [4]Mimica N, [5]Drmić S, [3]Skalny AV.

[1] *Institute for Research and Development of the Sustainable Eco Systems, Srebrnjak 59, 10 000 Zagreb, CROATIA, Tel (+3851) 243 0288*
[2] *University Hospital Centre Zagreb, Kišpatićeva 12, 10 000 Zagreb, CROATIA, Tel. (+38591) 202 7364,*
[3] *ANO Center for Biotic Medicine, Moscow, RUSSIA, Zemlyanoi val 46-48, 103604 Moscow, RUSSIA, Tel. (+7491) 681 27601*
[4] *University Psychiatric Hospital Vrapče, Bolnička cesta 32, 10 090 Zagreb, CROATIA, Tel. (+3851) 378 0678*
[5] *Neuropsychiatric Hospital "Dr. Ivan Barbot", Jelengradska 1, 44317 Popovača, CROATIA, Tel. (+38591) 702 265*
berislav.momcilovic@gmail.com, juraj.prejac@gmail.com, skalny3@orc.ru, skalny@microelements.ru, ninoslav.mimica@bolnica-vrapce.hr, stipe.drmic@gmail.com

Background: Today, lack of iodine is the most common endemic disease in both the developed European and underdeveloped third world countries, respectively. The currently available biological indicator of iodine status is urinary iodine that reflects the very recent iodine exposure, whereas the long term indicator of iodine remains to be identified.

Methods : We analyzed hair iodine (I_H) in an observational, prospective, cross-sectional, exploratory, epidemiological study involving 870 apparently healthy Croatians (270 ♂ and 600 ♀). The I_H was analyzed with ICP MS.

Results: The population (n_{870}) I_H median was 0.499 $\mu g \cdot g^{-1}$ (0.482 and 0.508 $\mu g \cdot g^{-1}$ for men and women respectively); there was no gender dependent difference between these I_H medians. To assess iodine deficiency, adequacy and excess, we studied the I_H deposition by analyzing the logistic sigmoid saturation curve of the median derivatives. We estimated the overt iodine deficiency to occur when the hair iodine concentration is below 0.100-0.150 $\mu g \cdot g^{-1}$. Then there was a saturation range interval of 0.100 to 2.000 $\mu g \cdot g^{-1}$ where the deposition of iodine in the hair was linearly increasing (r^2=0.994). Eventually, the sigmoid curve became saturated at about 2.000 $\mu g \cdot g^{-1}$ and upward, indicating excessive iodine exposure.

Conclusion : Hair is a valuable biological indicator tissue for assessing the long term iodine body status. We propose the adequate iodine status to correspond with the I_H concentrations of 0.650-1.250 $\mu g \cdot g^{-1}$ what agrees with the observed 60-80% hair iodine saturation range.

SPECIATION OF ALUMINUM-FLUORIDE COMPLEXES IN SOLUTIONS BY CAPILLARY ELECTROPHORESIS ELECTROSPRAY IONIZA TION MASS SPECTROMETRY

Daisuke Nakarnoto1, Tatsuya Urabe2, and Miho Tanaka 1

1 *Graduate School of Marine Science and Technology, Tokyo Univ. of Marine ScL and Tech., daiske-nakamoto@kzf.biglobe.ne.jp, mihotnk@kaiyodaLac.jp*

2 *RIKEN, t-urabe@riken.jp*

1. In recent years, hydrofluoric acid (HF) is used for surface microfabrication of glass. As a result, a large amount of fluoride-containing effluent has been discharged. Fluoride ion (F^-) has toxicity at high concentration, therefore, a co-precipitation method with aluminum-hydroxide ($Al(OH)_3$) is used for removing fluoride ions from effluent in many countries. However, chemical interaction between fluoride and aluminum ions is still not clear. To resolve this, an analytical method that provides chemical structural and ionic charge information on aluminum complexes should be required.

2. Electrospray lonization Mass Spectrometry (ESI-MS) provides structural information on aluminum complexes in solution, but cannot provide accurate ionic charge information. Therefore, capillary zone electrophoresis (CZE, CE) was connected on-line to ESI-MS for an ionie charge separation method by a customized CE-ESI interface.

3. Sample solutions were prepared by aluminum nitrate and sodium fluoride. The concentration of aluminum was fixed to 2.5 mM and [F]/[AI] ratios were varied from 0.5
to 2.0. Formic acid at a concentration of 5 mM was used as a background electrolyte (BGE) for CE. Ali solutions were adjusted to pH 3.0 ± 0.1 with nitric acid.

4. Three separated peaks were observed having retention times: 5, 6, and 8.5 min. CE separates chemical species depending mainly on their ionie charge differences, therefore, the species that corresponds to the three peaks were assumed to have different ionic charges in the sam pie solution However, from observed mass spectra, they were identified as singly charged species: [Al(OH)2 n(H20)]+, [AIF(OH) n(H20)]+, and [AIF2 n(H_2O)]+, respectively. Generally, free aluminum ions (AI3+) are affected by the deprotonated reaction on ESI gas-phase and $Al(OH)_2^+$ are generated. From these results and chemical equilibrium calculation, observed species in this study were considered as AI3+, AIF2+, and AIF_2^+ originally in the sample solution. In addition, the peak intensity ratio of AI3+, AIF2+, and AIF2+ observed by CE-ESI-MS were in good accordance with the abundance ratio calculated by chemical equilibrium theory, showing that CE-ESI-MS can be a useful probe for speciation of aluminum complexes.

1-25

THE GENDER DIFFERENCES IN ELEMENTAL CONTENT OF HIGH SCHOOL STUDENT'S HAIR

Baranova O.V.

Russia, Orenburg, Orenburg State University - baranovaov@yandex.ru

It is known, that human hair is a good indicator of some macro- and trace elements accumulation in the body. Also, the significant difference in elemental hair content between healthy males and females was shown. In our investigation 199 students of both sexes from Orenburg State University were observed and their hair samples were collected and analyzed by ICP-MS. We found the significant gender differences in hair content (Ca, Mg, I, Cu, Ni were higher in females, but K, Na, fe, Mn, Cr, Sr, Li, Al, Cd, Pb – in males). There were no gender differences in hair content of P, Zn, Co, Se, As, Sn, Hg. In both sexes there was very low hair Se content. The soil Se deficiency typical for Orenburg region. Also, we hypothesized, that the sulphur-rich natural gas extraction in Orenburg gas factory can be a reason of Se deficiency in soils, plants and some animal products, because of S-Se antagonistic interrelationships in biological systems. Thus, according to our data, Ca, Mg, K, Na, Fe, Cu, I, Mn, Cr, Pb, Cd and possibly Al, Li, Sr can be the elements with sex dependent mechanism of metabolic regulation in pooling hair growth. P, Zn, Se, Co, Sn, Hg, As hair content can be more dependent on ecological, but not gender influence.

1-26

DIURNAL SALIVARY MAJOR TRACE ELEMENT CONCENTRATIONS IN HEALTHY WOMEN

Ivan V. Radysh, Dmitry V. Brunin

Department Management of nursing, People's Friendship University of Russia, Moscow. E_mail: iradysh@mail.ru

Elemental balance of the human organism can fluctuate significantly depending on the genetic, temporal, biosocial and climatic factors. However, the greater value for the cyclic rearrangement of the female organism plays major and trace elements.

The aim was to study the daily variations of major and trace elements in whole saliva of the healthy women in different phases of the menstrual cycle.

The whole saliva samples of 55 healthy women aged 20-35 years were investigated during different phases of the menstrual cycle. Concentration of the following 16 chemical elements in the samples was determined: Al, B, Ca, Cu, Cr, Fe, K, Mg, Mn, Na, Ni, P, Pb, Se, Si, Zn (mg/g). Analytical determination has been carried out using ICP-AES and ICP-MS methods.

Results of researches have shown, that the maximum concentration Al, B, Ca, Cu, Cr, Mn, Na, Si, Zn and Ni was observed in the morning, and K, Mg, P, Fe, Se and Pb - in the evening ($p < 0{,}001$). The concentration B, Ca, Cu, Mg, Mn, Na, Se, Si, Zn in whole saliva was significantly higher ($p < 0{,}001$) in the mid-follicular phase, and Al, Cr, Fe, K, Ni, P and Pb - in the mid-luteal regardless of the menstrual cycle phase.It was found that the values of the salivary ratio Na/K was significantly higher in the morning and the mid-follicular phase. According to our data there is a simultaneous increase in the levels of trace elements copper and zinc in the mid-follicular phase and in the morning, which could confirm the activation of the antioxidant system during this period, menstrual cycle, and time of day. The Spearman range correlation analysis showed that between the values of the concentration zinc and copper in mid-follicular phase was the direct correlation between the morning ($r = 0{,}73$; $p < 0{,}001$), and in the evening - ($r = 0{,}55$; $p < 0{,}001$). Thus, we found that the concentration of the salivary major and trace elements in healthy women have the distinct diurnal and monthly rhythmicity.

4. Molecular biology

1-27

SIMILARITIES AND DIFFERENCES IN IL-6 AND TNF-A PRODUCTION INDUCED BY THE HUMAN γ-GLOBULIN METAL COMPLEXES

M.A.Apresova, I.E.Efremova, A.A.Babajanz, S.B.Cheknev.

N.F.Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia.
e-mail: cheknev@gamaleya.org

As it was found in our previous investigations, the metal complexes of human serum γ-globulin formed with copper or zinc ions did induce and regulate production of IL-1β by human peripheral blood cells (PBC).

Because of multiple ways connecting synthesis of proinflammatory cytokines in the response to antigen or mitogen stimulation, this work was performed with the purpose to study dynamics of IL-6 and TNF-α production by human PBC in presence of human serum γ-globulin metal complexes formed with copper or zinc ions.

The complexes were obtained after incubation of human serum γ-globulin (ICN) with the salts of copper or zinc at 37°C under the spectrophotometric control of the protein state and concentration (PU 8730 UV/VIS, Philips), two steps ultrafiltration of the samples in Ultracel-30K (Amicon) at 1700 g, restoration of the proteins in 0.15 M NaCl, and determination of the quantities of copper or zinc bound per one protein molecule.

Normal human PBC were incubated in the culture conditions with 0.5 μg/ml of the complexes tested for 24, 48 or 72 hrs.

The presence of IL-6 and TNF-α in the culture medium was measured by the ELISA Processor II (Behring). Test-systems of ZAO Vector-Best Europe (Russia) were used.

It has been shown that in presence of γ-globulin metal complexes, of the control proteins and of the copper or zinc used alone, normal human PBC produced up to 2.0 ng/ml of IL-6 and up to 140 pg/ml of TNF-α.

The complexes of γ-globulin with both copper and zinc ions exhibited higher activity in induction of both IL-6 and TNF-α production at 48 and 72 hrs of the cells incubation compared with their protein and cation controls. At the same time, in the early cell response (24 hrs of observation) the complex with zinc exerted the properties of both IL-6 and TNF-α inducer, whereas the complex with copper demonstrated induction of TNF-α production and inhibition of IL-6 synthesis, similarly to the induction of IL-1β.

It could be suggested that γ-globulins modified by chelating copper or zinc ions may serve as inducers and regulators of IL-6 and TNF-α production by human PBC.

1-28

THE PROTEINS OF HUMAN SERUM γ-GLOBULIN FRACTION TRANSFORMED WITH METAL IONS CHELATING – AS REGULATORS OF IMMUNE CELL ACTIVITY

SB Cheknev, IE Efremova, MA Apresova, EN Yushkovets, AA Babajanz.

N.F.Gamaleya Research Institute of Epidemiology and Microbiology, Moscow, Russia.
e-mail: cheknev@gamaleya.org

At physiological levels the immune cell activity is regulated, as well known, by multiple innate mechanisms including the effects induced by interaction of circulating antibodies with Fc receptors (FcR) of the cells, on the one hand, and the cell functions depending on the presence of metal ions in the cell microenvironment, on the other hand.

In spite of intensive study of the signal transduction pathways dealing the activation of FcR or with action of trace metals, possibility for chelating metal ions by the antibodies that might change a conformation of the proteins and thereby shift effector functions of such "newly designed" antibodies in cell activity regulation through FcR remains undescribed yet.

Using conditions similar to the physiological ones, we have obtained original metal complexes of human serum γ-globulin formed with copper or zinc ions, and have studied their properties in the culture of normal human peripheral blood cells (PBC).

The complexes were found to induce production by PBC of IFN-α, IFN-γ, IL-1β, IL-2, IL-6 and TNF-α, as well as to regulate production of IL-8, IL-10 and IL-18.

Three types of cell responses could be detected in presence of the complexes studied. Production of IFN-α, IFN-γ and IL-2 by human PBC was enhanced by the copper complex and decreased by the zinc proteins. The synthesis of IL-1β and IL-6 was augmented in presence of the zinc complex and reduced by the copper proteins. Concentrations of TNF-α, IL-8, IL-10 and IL-18 in the PBC culture medium were increased in presence of both copper and zinc proteins.

The data obtained allow to assume ability of γ-globulins transformed by binding the copper or zinc ions to impact into Th1/Th2 immunogenesis through direct effects on Th1 or Th2 cytokine production, as well as on the functions of monocyte/macrophages and dendritic cells which may be induced or inhibited depending on the transforming metal.

Such regulatory metallic complexes of γ-globulin seem to present a natural fraction of normal human serum proteins.

1-29

CADMIUM AND 5-FLUOROURACIL EFFECTS ON MCF-7 CELLS: MORPHOSTRUCTURAL STUDIES AND GENE-PROTEIN EXPRESSION

Asara Y.(1),Marchal JA(2), Boulaiz H.(2), Tolu P. (1), Farace C.(1), Peruzzu A(1), Montella A(1), Madeddu R.(1)

(1) Dept. of Biomedical Sciences, University of Sassari, Sassari, Italy
(2) Dept. of Human Anatomy and Embriology, University of Granada, Granada, Spain

Aim: Cadmium (Cd) is considered as one of the most dangerous metals, in particular for men's health. 5-fluorouracil (5-Fu) is an important antimetabolite in chemotherapy protocols for solid tumors. The MCF7 human breast cancer cell line had been used as an excellent cellular model. We analysed the correlation between MCF-7 ultrastructural alterations, Aetin and Tubulin cytoskeleton proteins and gene and protein expressions.

Methods: MCF-7 were treated with Cd (5 µM, 20 µM, 40 µM) and 5-FU (1.5 µM), for 24h and 48h, for TEM. SEM and Immunofluorescence. White for gene and protein expression MCF-7 were treated with the Ic 50 (Cd 3 µM. and 5-FU 1.5 µM) for 6h, 24h. 48h. Gene expression of Bel2, Bax, Caspase 3, Caspase 8, Caspase 9. C-Mye, Cyclin A 1, Cyclin 01, was studied with qRT -PCR, proteins Bel-2, Caspase 3, Caspase 8, Caspase 9, C-Myc, Cyelin-01, Cyclin A 1 were analyzed with Western Blot.

Results: in Cd treated cells we observed an intense metabolic activity with altered mitochondria and alterations in Actin distribution while Tubulin branched out throughout the cytoplasm.

5-FU-treated cells showed cytotoxicity signs with the presence of pore-like in the membrane cell and degeneration of cytoplasm and cell nuclei. The addition of 5-FU to the Cd treated cells did not induce significant structural changes in comparison with the Cd treated cells.

Genes and proteins expressions were significant at 24 hours. 5-FU+Cd treated cells had an increase of anti-apoptotic expression of Bcl-2, both Cyclins and C-Myc inverse correlation between the Cyclin 01 and A 1, with increased expression of 01. The results of the proteins were concordant with those of gene expression.

Conclusion: Our results suggest that Cd can prevent from the cytotoxic effects of 5-FU we used in our experiments and in traditional breast cancer therapy.

5. Epidemiology

1-30

WHOLE BLOOD SELENIUM LEVELS IN HEALTHY ADULTS FROM THE WEST OF ALGERIA

Dennouni Nouria[a], Harek Yahia[b] and Attar Tarik[b]

[a] *Laboratoire antibiotiques, antifongiques, physico-chimie, synthèse et activité biologique, Département de Biologie, Université de Tlemcen, Algeria*
E.mail: dennouria@yahoo.fr
[b] *Laboratoire d'électrochimie et chimie analytique. Département de Chimie, Université de Tlemcen, Algeria*

The purpose of the study was to assess whole blood selenium level of 300 healthy adults living in four selected areas of the west of Algeria. Selenium was measured using differential pulse cathodic stripping voltammetry (DPCSV); the detection limit of selenium was 29.20 µg/L. The mean and standard deviation of whole blood levels was 85.65±21.60 µg/L ranging between 30.9 and 144.04 µg/L. This concentration did not vary significantly (P>0.05) in relation to the sex of the subject, with concentrations of 87.75±21.35 µg/L in men and 83.95±21.62 µg/L in women group. Individuals older than 60 years had a whole blood selenium concentration significantly lower than the rest of the population. However, the measured selenium concentrations in the residential areas were not statistically different (P>0.05). A total of 32 (10.7%) individuals exhibited whole blood selenium level below 60 µg/L.

These results are similar to those of some European countries but are much lower than data observed in USA or seleniferous regions.

GENDER DIFFERENCES IN BLOOD CADMIUM CONCENTRATIONS AND THEIR RELATIONSHIP WITH SMOKING

Díaz Díaz MA*, Castillo Pérez C*, Trasobares E*, Fuentes M**, Martínez MJ***, González-Estecha M*

**Laboratory Medicine Department. Trace Element Unit. **Research Unit. Hospital Clínico San Carlos. Madrid*
****Universidad Politécnica de Cartagena*
Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC). Madrid (Spain).

Background. Exposure to cadmium is a public health problem due to broad exposure among the general population. The objective of this study is to analyze blood cadmium levels in a hospital working population and their association with smoking and gender differences.

Methods. 395 employees were recruited (64 men and 331 women), with a mean age of 47 (SD:10.9). Blood cadmium levels (µg/L) were measured by electrothermal atomic absorption spectrometry.

Results. The median of blood cadmium was 0.29 µg/L (IQR 0.18-0.50), with women having higher levels (0.31; IQR 0.20-0.51) than men (0.20; IQR 0.11-0.40; p=0.007).

By way of a multiple linear analysis adjusted for age, sex, ferritin, hemoglobin and smoking, women had a mean blood lead level 1.24 times higher than men (p=0.005). The median blood cadmium of smokers (0.83 µg/L) was higher than that of ex-smokers (0.31 µg/L), which in turn was higher than that of subjects who had never smoked (0.21 µg/L); this difference was statistically significant (p<0.001). An association was observed between blood cadmium levels and the number of cigarettes inhaled (smokers r= 0.620; p< 0.001; ex-smokers r=0.265; p=0.001), as well as with the time elapsed since quitting smoking (r=-0,200; p=0,017). Passive smokers had higher blood cadmium (0.24 µg/L) than those who were not (0.20 µg/L; p=0,035). By way of a multiple linear analysis, adjusted for age, sex, ferritin and hemoglobin levels, passive smokers had , on average, 17% higher cadmium levels (relative rate 1.17; CI 95%:0.9-1.4; p=0.088).

Conclusions. Women had higher blood cadmium levels than men. Blood cadmium levels are related to smoking. More studies are needed in order to confirm the higher blood cadmium findings in passive smokers.

BLOOD CADMIUM, SMOKING BEHAVIOR AND OTHER ASSOCIATED FACTORS AND THEIR RELATIONSHIP WITH ESTIMATED GLOMERULAR FILTRATION RATE

Diaz -Díaz MA*, Castillo Pérez C*, González-Estecha M*, Trasobares E*, Fuentes M*, García-Donaire JA**, Abad-Cardiel M**, Martell Claros N**.

**Laboratory Medicine Department. Trace Element Unit. **Hypertension Unit.*
Hospital Clínico San Carlos. Madrid (España)
Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC). Madrid (Spain).
Email: montse@cmpx.net

Background. Low cadmium levels (0.43 µg/L) have been associated with nephrotoxicity and cardiovascular disease among the general population. The aim of this study is to evaluate the association between blood cadmium concentrations and the estimated glomerular filtration rate (eGFR) adjusted by age, smoking behavior and other associated factors in a hospital working population.

Material and Methods. We recruited 395 employees (64 men and 331 women) with a mean age of 47.3 (SD: 10.9) years old who were administered the PESA® exposure questionnaire. Blood cadmium was measured by electrothermal atomic absorption spectrometry. eGFR was assessed using the abbreviated CKD-EPI formula.

Results. 6.7% of the subjects had hypertension, 20.6% were smokers and 3.2% were diabetics. The median of blood cadmium was 0.29 µg/L (IQR: 0.18-0.50) and the mean eGFR was 78.95 mL/min/l.73 m² (SD: 11.86). Considering an eGFR cutoff point of 75 mL/min/l.73 m², the participants below the cutoff had a median of cadmium of 0.33 µg/L (IQR: 0.20- 0.52) and the ones over the cutoff had 0.27 µg/L (IQR: 0.16- 0.45); these differences were statistically significant (p=0.028).

We adjusted a logistic regression model in order to estimate the effect of age, tobacco, sex, ferritin, LDL-C, hypertension and uric acid. Patients with blood cadmium≥30 µg/L were 1.7 times more likely to have a lower eGFR that those with blood cadmium <0.30 µg/L (OR= 1.7 (95% CI 1.02-2.82;p=0.038). However, when we introduced blood lead and mercury levels in this mathematic model we did not observe this statistical signification (OR=1.55; 95% CI 0.93-2.61;p=0.09) due to the important interaction between mercury and the eGFR (p=0.03).

Conclusions. We did not observe any influence of blood cadmium levels on the glomerular filtration rate in the studied population. More studies are needed in a population with higher exposure to cadmium in order to be able to verify the role of low cadmium concentrations on kidney function.

6. Sport

1-33

TRACE ELEMENTS AND HORMONAL VARIATIONS LN SERUM DURING AN INCREMENTAL EXERCISE IN ENDURANCE ATHLETES

Gonzàlez-Haro, C.(1), Soria, M.(1), Ansén, M. A.(1), lrïigo, C.(2), Calvo, M. L.(2) & Escanero, J. F.(1)

(1) Pharmacology and Physiology Department. School of Medicine. University of Zaragoza, Spain;
(2) Clinical Biochemistry Service. Hospital Miguel Servet, Zaragoza, Spain.

Background: It has been suggested that serum strontium levels could be regulated by serum calcium levels, furthermore calcitonin was suggested to be involved in zinc regulation, for that reason it was proposed the shared hormonal regulation theory for trace elements, although the discovery of the genetic regulation meant the abandonment of hormonal regulation theory.

Aim: To assess the effect of relative exercise intensity effect on some trace elements and serum hormones levels in euhydrated endurance athletes.

Methods: 27 endurance athletes carried out a warm-up on cycloergometer of 10 min at 2.0 W-Kg-1 and increments of 0.5 W-Kg-1 until exhaustion. Oxygen uptake (V02), trace elements (Zn, Se, Mn, Co and Mg) and hormonal (TSH, Glucagon, Catecholamines, PTH, Cortisol and Insulin) serum levels were measured at rest, at the end of each stage and at minutes 3rd, 5th and 7th post-exercise. Urine density and pH were analysed before and after the test, subjects drank water ad libitum. Fat oxidation (FATox), carbohydrate oxidation (CHOox), energy expenditure from fat (EEFAT), energy expenditure from CHO (EECHO), total EE were estimated by means stequiometric equations. All parameters were compared in time by means repeated measures ANOVA. Pearson's correlation was performed to assess the relationship between variables. Significance level was at P<0.05.

Results: Euhydration of subjects was controlled (drank: 842 [1197 mL of water, no significant differences neither urinedensity: 1.014 10.004 YS. 1.014 10.004 g-cm-3 nor urine pH: 5.90 10.75 vs, 5.98 10.77 pH). No significant differences were observed in serum trace element levels respect to the intensity of exercise (W-Kg-1). During the progressive exercise TSH, glucagon, catecholamines, PTH and cortisol increased (P<0.05-0.001); insulin decreased (P<0.01); and calcitonin, aldosterone and PRL did not change. Furthermore, no significant correlations between trace elements respects with the hormones studied were found, neither between trace elements respects to V02, FATox, CHOox, EEFAT, EECHO and EET was established.

Conclusions: In euhydrated endurance athletes, 1. Serum trace element levels do not vary with the relative intensity of exercise, 2. Serum hormone responses have no relationship with serum trace elements concentration during a progressive exercise until the exhaustion.

INORGANIC ELEMENTS DETERMINED IN BLOOD OF ELITE RUNNERS USING NAA

C.B. Zamboni 1, L. Kovacs 1, L. A. S. Nunes 2, T. F. Lourenço 2, D. V. Macedo 2

1 *Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN/SP -Av. Professor Lineu Prestes 2 242 05508-000 São Paulo, SP, Brazil*
czamboni@ipen.br - lukovacs@gmail.com
2 *Universidade Estadual de Campinas, UNICAMP, Brazil - Laboratório de Bioquímica do Exercício – LABEX - Cidade Universitária 13083-970 - Campinas, SP - Brazil - Caixa-Postal: 6109*
lazaroalessandro@yahoo.com.br - mailto:Thiago_fl@yahoo.com.br denisevm@unicamp.br

In the last decade there was a growing interest in the athlete's health with a focus on continuous biochemical evaluation (in serum and urine) and controlled diet. Nowadays is recognized that intensity and duration of the physical training can provoke metabolic alterations in blood, mainly in the content of some ions. Recently elements of clinical relevance (such as Ca, Cl, Mg, K and Na) in blood of amateur and elite runners (long distance runners) were analyzed and a comparison with subjects of same gender and age but not involved with physical activities revealed significant differences among them, mainly between control and elite groups. In this study we intend to continue this analysis, to complement the blood investigation, performing measurement to determine Fe, I, Rb and Zn using NAA. Ten athletes (6 male and 4 female), age 18 to 36 years, participated of this study. The blood collection was performed at LABEX (SP, Brazil) and the samples were irradiated in the nuclear reactor (IEA-R1, 3-4.5MW, pool type) at IPEN/São Paulo - Brazil.
These data can be considered for the preparation of balanced diet, to evaluate the performance of endurance athletes during the period of competition preparation and also to propose new protocols for clinical evaluation

1. Ageing

INTERACTION OF STRONTIUM IONS WITH BONE MINERALIZATION AND APATITE CRYSTALS

Georges Boivin, Audrey Doublier, Sébastien Rizzo, Delphine Farlay

INSERM, UMR 1033, F-69372 Lyon, France
Université de Lyon, F-69008 Lyon, France
georges.boivin@inserm.fr
audrey.doublier@gmail.com
sebastien.rizzo@inserm.fr
delphine.farlay@inserm.fr

Strontium ranelate (SrRan, 2g/day) is a treatment of osteoporosis (OP) with a broad spectrum of safety and efficacy as it increases bone mineral density at all sites and reduces the risks of both vertebral and nonvertebral fractures (including hip) in a wide variety of patients. Strontium (Sr) is known to interact with bone mineral and apatite crystals. At the tissue level, up to 5 years of treatment, secondary mineralization is maintained at a normal range. Sr is heterogeneously distributed in bone tissue: absent from older bone, Sr is exclusively present in younger bone formed during treatment. The extention of bone Sr area increases with duration of treatment but the focal Sr content remains constant (1.66±0.32 weight % in Sr bone area). At the crystal level, incorporation of Sr within apatite crystals represents a maximum of 0.5 Sr ions per 10 calcium ions after 3 years of treatment; thus, the mean rate of substitutions of calcium by Sr is low (4.5%). Variables reflecting crystal and unit cell characteristics (crystallinity, apparent length and width/thickness of crystals, interplanar distances, and lattice parameters of unit cells) are not influenced by the presence of Sr. Values are similar to that measured in patients receiving a placebo for 3 years. These results, in association with a rapid clearance of Sr from bone, suggest that Sr mostly interacts with the hydrated layer rather than to be trapped for a long time in the crystal lattice. Besides, in rats, SrRan improves mechanical properties (hardness and ability of bone tissue to dissipate energy) at the tissue level in hydrated conditions while bone formation is stimulated. It is, therefore, hypothesized that the presence of Sr in bone matrix may modify it in relation to the hydration state of the bone with a positive consequence in local and global mechanical behavior. In conclusion, the quality of bone mineral and apatite crystals is maintained, even after a prolonged administration of SrRan.

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BRAZIL NUT: A WAY TO IMPROVE SELENIUM STATUS OF ELDERLY WITH MILD COGNITIVE IMPAIRMENT

Bárbara Rita Cardosoa*, Rafael Barofaldi Buenoa, Wilson Jacob-Filhob, Silvia Maria Franciscato Cozzolinoa

*a Faculty of Pharmaceutical Sciences, University of São Paulo (USP), São Paulo, Brazil; b Division of Geriatrics, University of São Paulo Medical School (USP), São Paulo, Brazil; *barbaracardoso@usp.br*

Introduction: Studies have shown that various antioxidants are decreased in different age-related degenerative diseases and thus, oxidative stress would have a central role in the pathogenesis of many disorders that involve neuronal degeneration. Thus deficient status of antioxidants is associated with cognitive decline and risk of dementia. We previously showed that Alzheimer's disease patients have a deficient selenium (Se) status, and in this study we aimed to evaluate if daily consumption of Brazilian nut would have a positive effect upon Se status in elderly with mild cognitive impairment (MCI).

Materials & Methods: Eighteen elderly with MCI (76.6±4.2 y old), from Hospital das Clínicas of the São Paulo University Medical School (São Paulo, Brazil) were studied. They were randomly divided in two groups: Brazilian Nut group (BN, n=7) received one nut (around 5 g, averaging 57.75 µg Se/g) a day for 6 mo, and Control group (CG, n=11) did not receive anything. Se concentrations in the nuts and in plasma were determined by atomic absorption spectrophotometry with hydride generation (Hitachi, Z-500).

Results: Plasma Se was not different between groups in baseline (BN=53.78±15.58 µg Se/L; CG=52.09±23.36 µg Se/L; $p>0.005$). However, after 6mo treatment, Se plasma was significantly higher in BN (263.71±80.43 µg Se/L; $p<0.001$), while CG decreased plasma Se (29.90±13.67 µg Se/L; $p<0.05$).

Conclusions: The data revealed that the consumption of only one Brazil nut a day (5 g) during 6 mo was effective to increase the Se concentration in plasma. Thus, daily Brazil nut intake may improve antioxidant status of MCI people, thereby reducing the risk of conversion from MCI to dementia.

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ROLE OF ZINC IN DIAGNOSTICS AND ETIOLOGY OF PROSTATE CANCER

Vladimir Zaichick

Radionuclide Diagnostics Department, Medical Radiological Research Centre, Russia
vezai@obninsk.com

The Zn content in normal (N, n=37), benign hypertrophic (BPH, n=43) and cancerous tissues (PCa, n=60) of the human prostate gland were investigated by instrumental neutron activation and by energy dispersive X-ray fluorescent (EDXRF) analysis. Additionally, EDXRF was used to estimate Zn concentration in expressed prostatic fluid. Groups of patients suffering from chronic prostatitis (ChP), BPH and PCa consisted of 28, 28 and 13 men, respectively, were examined. The control group included 22 healthy volunteers.

It was found that tissue content of Zn is significantly low in cancerous tissues as compared to normal and benign hypertrophic tissues. Almost no difference was found between the Zn concentration for ChP, for BPH, and for N prostatic fluid. Prostatic neoplasm resulted in significant decrease of Zn level. Finally, we propose to use the estimation of Zn mass fraction in a needle-biopsy core or Zn concentration in a sample of expressed prostatic fluid as an accurate tool to diagnose prostate cancer.

It was also found age-dependent exponential increase of Zn mass fraction in normal prostate tissue and almost constancy of Zn concentration in prostatic fluid. In order to clarify the age-related histological changes in nonhyperplastic adult prostate glands, a quantitative morphometric analysis was performed. The percent volumes of the glandular lumen increase from the third to the fifth decade and reach a maximum at about 50 years of age. This suggests that stasis of the prostatic fluid develops from 30 to 50 years of age. The intracellular zinc concentration increased very fast after the age of 40, and by 50 to 55 years old it was over 15-fold higher, on the average, than the level typical of men aged 20 to 30. Thus, a drastic increase in the incidence of clinical prostate cancer in men over 50 can be related to an excessively high intracellular content of zinc.

CHROMIUM AND COBALT CONCENTRATIONS IN PATIENTS WITH METAL-ON-METAL HIP REPLACEMENTS: A COMPARISON BETWEEN TWO DIFFERENT BEARINGS

*Palazón-Bru I, **Castell A, *López Lozano L, ***Moro E, ***Otero J, *González-Estecha M, ****de Pedro Moro JA

**Laboratory Medicine Department. Trace Element Unit*
***Traumatology Department. Hospital Príncipe de Asturias *** Traumatology Department. Fundación para la Investigación Biomédica, Hospital Clínico San Carlos.Madrid (Spain)*
***** Traumatology Department. USAL. Salamanca (Spain)*
Email: irene_pb@hotmail.es

Background. There has been growing concern regarding the systemic and local effects of metal ions released from metal-on-metal hip replacements. Birmingham Hip resurfacing (BHR) and Acetabular System Resurfacing (ASR) are two models of metal-on-metal hip arthroplasty. A higher Chromium (Cr) or cobalt (Co) concentration than 7 µg/L in blood has been found in symptomatic patients. The aim of this study is to compare the Cr and Co concentrations released from ASR and BHR.

Material and methods. We studied 17 patients (16 male and 1 female) with a mean age of 49.3 (SD 8.1) for BHR and 23 patients (15 male and 8 female) with a mean age of 54.7 (SD 12.0) for ASR. Serum Cr concentrations were measured by Electrothermal Atomic Absorption Spectrometry; blood and urine Co and urine Cr concentrations by Inductively Coupled Plasma Mass Spectrometry.

Results. Medians (IQR) of serum Cr levels were not significantly different (p= 0.7) in patients with BHR:1.25 µg/L (0.9-4.7) compared with patients with ASR:2.30 µg/L (0.9-8.7).

Medians (IQR) of blood Co levels were not significantly different (p=0.5) in patients with BHR: 1.52 µg/L(1.5-4.3) compared with patients with ASR 1.45 µg/L(1.0-8.0).

Medians (IQR) of Co and Cr levels in urine in ASR patients were 5.10 µg/L (3.5-48.0) and 2.70 µg/L(1.4-15.0), respectively. For Co, a positive correlation was found between blood and urine for ASR (r=0.701; p=0.001) as well as for Cr between serum and urine (r=0.737; p<0.001).There was no association (Co: p=0.22; Cr: p=0.07) between the type of bearing and a higher metal concentration than 7 µg/L (Co: 27.3% ASR vs 7.7% BHR; Cr: 26.1% ASR vs 0% BHR).

Conclusions. The metal concentrations in urine for ASR are directly proportional to the metal level in blood or serum. Furthermore, although there were no differences between bearing type and a higher metal level, more patients with ASR than BHR bearings had high metal levels.

INVESTIGATIONS ON THE MECHANISM OF MANGANESE-INDUCED INJURY OF DOPAMINERGIC NEURONS

Katharina Fernsebner (Katharina.Fernsebner@helmholtz-muenchen.de), Bernhard Michalke

Helmholtz Zentrum München, Analytical Biogeochemistry, Ingolstädter Landstr. 1, Munich, Germany

Manganese (Mn) is an essential trace element and therefore important for abundant physiological processes serving as cofactor of several enzymes, among others the acetylcholinesterase (AChE). On the downside, overexposure to Mn can lead to the degeneration of dopaminergic neurons inducing a complaint called manganism. Various investigations on the transport route and species of Mn into the brain have been carried out, but the mechanism of neurodegeneration is rather complex and therefore still unclear.

The aim of this study is therefore to find out the relation between Mn-species and neurotransmitters, acetylcholinesterase activity and misbalances between other trace elements (like Fe, Cu or Zn) to clarify the mechanism which is leading to the Mn-dependent neurodegeneration.

We therefore applied a first feeding trial in rats for four weeks to cause a chronic overexposure. During that time, control rats were fed with casual fodder and test rats were supplemented with Fe-deficient and Mn-rich fodder (100 mg/Kg). Brain, liver, kidney and serum were analyzed by ICP-AES or ICP-MS and Mn speciation was performed by SEC-ICP-MS. For determination of total contents of Mn, Fe, Cu, Zn, both chemical digestion and extraction of brain, liver and kidney [1] were carried out, whilst extracts or serum were used for Mn-speciation and determination of AchE activity.

The determination of total content of Mn showed only little difference in the brain, whereas in serum it was elevated appr. 50% compared to control. Regarding AchE activity, it was 10% lower in test rats only in serum but showed no significant difference in brain samples.

To obtain major differences between control and test rats, we will apply a second feeding trial, where Mn-concentration in fodder of test rats will be elevated to 500 mg/Kg and feeding time will be extended to 8 weeks. In parallel we will apply i.v. treatment of rats with $MnCl_2$ according to Diederich et al. [2] to compare oral intake of Mn with the systemic absorption.

Results of further experiments will be presented comprising FT-ICR-MS to check the involvement of other neurotransmitter pathways (e.g. glutamine) and LA-ICP-MS of brain sections to localize the supposed trace elements in the brain.

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1-40

TRACE ELEMENTS IN HUMAN BRAIN: AGE-RELATED CHANGES IN DIFFERENT ANATOMICAL REGIONS AND CHANGES RELATED WITH NEURODEGENERATIVE PROCESSES

Agostinho Almeida 1, Patricia Ramos 1, Nair Pinto", Ricardo Mendes", Agostinho Santos"

1 REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Facultyof Pharmacy, University of Porto, Portugal. aalmeida@ff.up.pt
2 National Institute of Legal Medicine and Forensic Sciences, North Branch, Porto, Portugal. asantosinml@yahoo.com

One of the factors identified as potentially involved in the aetiology of neurodegenerative diseases (ND) are disturbances on the homeostasis of some trace elements (TE), namely, Cu, Fe, Mn and Zn in particular areas of the brain. The goals of this work were to study, directly in human brain samples: 1) the regional anatomie differences of TE levels wfthfn the brain; 2) the changes on TE levels in relation to ageing; and 3) the differences in TE levels between individuals with and without evidence of ND.

From each neurologically and psychiatrically healthy individual submitted to autopsy (n=44) the following 14 areas were sampled: frontal cortex; superior and middle temporal; caudate nucleus, putamen, globus pallidus; cingulated gyrus; hippocampus; inferior parietal lobule; occipital lobe; midbrain; pons; medulla; and cerebellum. Samples from individuals with previous diagnosis of Alzheimer's (n=2) and Parkinson's disease (n=1) were also collected. After microwave-assisted acid digestion of the samples, Cu, Mn and Zn were determined by ICP-MS and Fe by GFAAS.

Fe (mean±sd: 343±65 f.Jg/g) was the most abundant metal, followed by Zn (53±4 f.Jg/g), Cu (21±5 f.Jg/g) and Mn (1.3±0.4 f.Jg/g). It was found that distribution of TE within brain tissue is not homogeneous. Zn highest levels were found in hippocampus; hfghest levels of the other three TE were found in basal ganglia. Pons and medulla were the regions with lowest levels of the four TE studied. In specifie areas, TE levels proved to be age-related: it was observed a positive correlation for Fe, Mn and Zn but a negative trend for Cu.

When compared with non-diseased people of the same age sub-group, individuals affected by Alzheimer's disease showed significantly altered TE levels in regions mainly related to memory and learning. In a Parkinson's disease patient, high levels of Fe in brain areas related to motor and movement control functions were observed.

8. Pathologies

ALTERED LIPID PEROXIDATION AND ANTIOXIDANT VITAMINS STATUS IN TUNISIAN PATIENTS WITH NON SMALL CELL LUNG CANCER

Belkhiria MN1, Jeball MA1, Knani J2.

1 Laboratoire de Pharmacologie, Faculté de Médecine, Université de Monastir, Tunisie.
Noura.Belkhiria@fmm.rnu.tn
2 Service de Pneumologie, CHU Tahar Sfar, Mahdia, Tunisie.

Objective: The aim of this study was to investigate oxidative stress status in different stages and histological types of lung cancer.

Material and methods: In total, 58 patients with non small cell lung cancer and 81 controls were assessed. Lung cancer patients were divided into those with early stage or advanced stage disease. The tumour type was squamous cell carcinoma (SQCC) in 34 patients and adenocarcinoma (AC) in 24. Plasma levels malondialdehyde (MDA), ascorbic acid, retinol and α-tocopherol, were determined. **Results and Discussion**: The levels of MDA were significantly higher in patients with early stage disease (3.52 ± 0.86 µmol/l) than in controls (2.71 ± 0.8 µmol/l, $p < 0.01$) and in patients with advanced stage disease (4.50 ± 1.23 µmol/l) than in controls (2.71 ± 0.8 µmol/l, $P < 0.0001$). Plasma MDA levels were also significantly higher in patients with advanced stage disease than in those with early stage ($P < 0.01$). The levels of ascorbic acid in patients with advanced stage disease (47.9 ± 14.4 µmol/l) were significantly lower than controls (57 ± 17 µmol/l, $p < 0.01$). The retinol concentrations were significantly lower in the group with advanced stage disease (3.37 ± 0.57 µmol/l) than in controls (3.61 ± 0.60 µmol/l, $p < 0.05$). Alpha-tocopherol levels were significantly lower in the group with advanced stage disease (19.3 ± 3.5 µmol/l) than in the controls (22.8 ± 4.1 µmol/l, $p < 0.0001$). Plasma MDA levels were significantly higher in patients with SQCC (4.56 ± 1.36 µmol/l) than in those with AC (3.80 ± 0.84 µmol/l, $P < 0.05$).

Conclusion: The results suggest that with advancing stage of lung cancer, the levels of oxidative stress increase, while levels of antioxidant molecule decrease. Patients with SQCC have higher oxidative stress as reflected by higher levels of MDA.

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ZINC, DIABETES MELLITUS AND CARDIOVASCULAR DISEASE

Samir Samman PhD

Discipline of Nutrition & Metabolism, School of Molecular Bioscience, University of Sydney NSW 2006 Australia

Background – Recognition of the public health importance of zinc continues to expand. Impaired zinc homeostasis features prominently in Type 2 diabetes mellitus (DM) and cardiovascular disease (CVD) (1). The possibility that replenishment with zinc could provide an adjunct therapy for these and other chronic diseases is compelling.

Objectives – To evaluate the effects of zinc supplementation on recognized risk factors for CVD and DM in humans. A systematic review of the literature and meta-analysis were undertaken.

Outcomes – In relation to CVD risk factors, the findings from randomized controlled trials showed an overall lack of effect of zinc supplementation on plasma lipids. When the trials were assessed based on the underlying health status of the participants, zinc resulted in a clinically significant decrease in high-density lipoprotein (HDL) in healthy subjects (an undesirable response), but a significant rise in HDL in those with DM, indicating a decreased risk of CVD. In DM patients, zinc supplementation decreased glucose concentrations and %HbA1c, and in some intervention studies decreased systolic and diastolic blood pressure. These observations were supported by results from cross-sectional surveys and from a limited number of cohort studies.

Conclusions – Improved zinc status appears to affect favourably the cardio-metabolic profile in people with DM. Further understanding of the mechanisms that underpin the cellular transport, sensing, and distribution of zinc will help to explain the effects of zinc in humans.

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1-43

A STUDY OF COPPER INDICES IN PARKINSON'S DISEASE AND ESSENTIAL TREMOR.

Bain PG1, Sampson B2

1 Department of Neurosciences, Imperial College London, UK
2Trace Element Laboratory, Department of Clinical Biochemistry, Imperial College NHS Trust, UK; b.sampson@imperial.ac.uk

Objective: To examine the relationships between copper indices and age, age of symptom onset and disease duration in Parkinson's Disease (PD) & Essential Tremor (ET). Methods: Ninety-nine consecutive outpatients with PD and 56 with ET had blood drawn for measurement of serum copper (Cu) and caeruloplasmin (Cp). Serum percentage free copper was calculated: % Free Cu = [total Cu - Cu bound to Cp]/Total Cu.

Results: in ET mean serum Cu: 18.0 µmol/L, Cp: 0.34 g/L & % Free Cu: 11.4%. Females had higher Cu than males. There were significant positive correlations between age and Cu & Cp levels, and age of onset with Cp & % Free Cu. Partial correlations, adjusting for age & gender, showed positive correlations between age of onset with Cu & Cp (p=0.001 & p= 0.011 respectively) and negative correlations between tremor duration and Cu & Cp (p=0.001 & p= 0.011 respectively).

In PD mean serum Cu: 16.6 µmol/l., Cp: 0.32 g/L & % Free Cu: 9.85%. In PD there were no significant correlations between age, age of onset or disease duration and any of the copper indices, even after adjusting for age and gender.

Cu (but not Cp or % Free Cu) was significantly lower in PD than ET (p=0.026).

Conclusion: We demonstrate significant relationships in ET between serum Cu & Cp levels and age of onset and tremor duration. This relationship was not found in PD. An explanation for these findings is that Cu & Cp provide some temporal protection from manifesting ET.

1-44

ANTI-INFLAMMATORY AND ANTI-ULCEROGENIC ACTIVITY OF ZINC HYDROASPARTATE IN RATS

Magdalena Gawel1, Anna Lipkowska1, Tadeusz Librowski1, Gabriel Nowak1, 2

1Department of Radioligands, Faculty of Pharmacy, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Kraków, Poland; 2Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland; m.gawel.87@gmail.com

Zinc is an essential trace element required for the proper functioning of numerous proteins, cell proliferation and differentiation. Studies have demonstrated that zinc acts as an antioxidant and anti-inflammatory agent protecting mucosal cells against induced gastric ulcers in animal models. Non-steroidal anti-inflammatory drugs, though commonly used and usually well tolerated by patients, frequently cause gastrointestinal erosions and ulcerations. The study's aim was to evaluate the influence of single and multiple administration (p.o. and i.p.) of selected zinc compounds on the anti-inflammatory and ulcerogenic activity of ketoprofen. The experiments were carried out on rats, male albino Wistar (140-290 g). Anti-edematous activity was determined using carrageenan-induced hind paw edema test developed by Winter et al. The irritant action on the gastric mucosa was assessed macroscopically in accordance to Komatsu. The results show that the percent of edema reduction in rats depends on the doses used, duration of the experiment and route of administration. Zinc enhanced the anti-edematous activity of ketoprofen in all experiments. Some of the selected zinc compounds given alone without ketoprofen caused the significant reduction of the edema in comparison to the control groups. It was assessed that zinc alleviated gastrointestinal injuries caused by ketoprofen, however more research are being conducted to confirm the gastroprotective properties of selected zinc compounds.

1-45

MYOCARDIAL METALS AND THEIR RELATIONSHIP TO THE PARAMETERS OF OXIDATIVE STRESS AND CARDIOVASCULAR IMPAIRMENT

Premysl Mladenka1, Tomas Filipsky1, Jaroslava Vavrova2, Magdalena Holeckova2, Vladimir Palicka2 & Radomir Hrdina1

Charles University in Prague;
1 Faculty of Pharmacy in Hradec Kralove, Department of Pharmacology and Toxicology, Heyrovskeho 1203; 500 05 Hradec Kralove, Czech Republic
2 School of Medicine and University Hospital, Institute for Clinical Biochemistry and Diagnostics, SokolskÃ 581,50005 Hradec KrÃ lovÃ©, Czech Republic. Email: mladenkap@faf.cuni.cz

Myocardial trace metals, in particular selenium, copper and zinc, are essential components of human antioxidant enzymes. On the other hand, free iron or copper are able to catalyse propagation of reactive oxygen species. Calcium is necessary for many physiological functions of human cells but its excess is commonly found in cardiovascular diseases.

Thus the aim of this study was to analyse the relationship of myocardial metals with parameters of oxidative stress and cardiovascular system dys/function.

The data from 145 male Wistar:Han rats, previously published in original papers analysing effects of iron chelators on a model of acute myocardial infarction (AMI), were re-analysed in this study. A pathological state similar in many aspects to AMI in humans was induced in a ha If of these animais by administration of isoprenaline (100 mg.kg-1, s.c.). Animals were pre-treated with different iron chelators or solvents in order to mimic treated and non-treated population with/without cardiovascular disorder.

As expected, elevated calcium level in the myocardium was associated with cardiac impairment, e.g. release of cardiac troponin T, elevated heart rate and increased wet ventricles weight index. Moreover, a negative correlation between calcium and blood glutathione was found. Increased myocardial copper level was associated with elevated both systolic and diastolic blood pressures while there were no correlations or correlations with very low coefficient (SO.2) between parameters of cardiovascular dysfunction and myocardial selenium, zinc or iron concentrations. Concerning the relationship of oxidative stress with myocardial trace elements, positive relationships of zinc with vitamin C and glutathione peroxidase are of note.

Conclusively, it appears that changes in myocardial trace metals do not directly correspond to the acute cardiovascular dysfunction and oxidative stress status.

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FEATURES OF THE TRACE ELEMENT STATUS OF PERSONS WITH DIFFERENT TSH LEVELS

Notova S.V., Miroshnikov S. V., Alidzhanova I.E., Kiyaeva E.V., Bibartseva E.V.

Orenburg State University, Russia, 460018, Orenburg, prospect Pobedy, 13, E-mail: inhip@mail.ru

190 Orenburg State University male students took part in the study. All respondents were divided into two groups, depending on the values of TSH. Persons with high TSH level (2,6-4,0 mU / l) were included in 1st group, students with low TSH level (0.4-2.5 mU / L) were included in 2nd group. Study of the TSH levels, free T_4 and T_3 in serum was performed by enhanced chemiluminescence «AMERLITE». Hair samples were used to study the trace element status of the body. The elemental composition of hair identification was performed at the Center for Biotic Medicine (Moscow). More often the high TSH level was associated with increased hair macro elements (potassium, calcium, magnesium, sodium), conditionally essential elements (lithium, vanadium) and some essential chemicals (cobalt, chromium, manganese, iodine) and toxic aluminum. At the same time, persons with high TSH level have had lower rates of copper, selenium, and zinc. These elements are involved in the functioning of the thyroid gland and the metabolism of thyroid hormones.

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1-47

ZINC NUTRITIONAL STATUS AND ENZYMES ANTIOXIDANT ACTIVITY IN PATIENTS WITH RHEUMATOID ARTHRITIS.

Graziela Biude Silva1; Kátia Rau de Almeida Callou1; Isabela Saraiva de Almeida1; Larissa Bezerra Santos1; Daniel Feldman Pollak2; Silvia Maria Franciscato Cozzolino1

1 Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Science, University of São Paulo, São Paulo, Brazil.
2Department of Rheumatology, Faculty of Medicine, Federal University of São Paulo, São Paulo, Brazil.
E-mail: gbiude@usp.br

Studies show an increased oxidative stress in patients with rheumatoid arthritis (RA). Zinc is an essential micronutrient for human health and plays an important role as an antioxidant by the enzyme superoxide dismutase (SOD). Data in the literature on zinc status in these patients, as well as the enzymatic activity of SOD are scarce and controversial. The aim of this study was to evaluate the activity of SOD and glutathione peroxidase (GPx), and zinc status in patients with RA. 16 female patients were selected, aged between 19 and 69 years, diagnosed RA according to the criteria established by the American College of Rheumatology, which make clinical monitoring at the Hospital São Paulo. The exclusion criterias included the presence of other diseases and intake of medicine that could interfere with the zinc biochemical profile. The zinc concentrations in plasma and erythrocytes were determined by atomic absorption spectrometry flame. The enzymatic activity of erythrocyte SOD and GPx in whole blood were made on a biochemical analyzer using commercial kits. Results were expressed as mean and standard deviation. The mean age of participants was 52.1±14.5 years. The mean plasma zinc concentration was 52.4±7.5 µg/dL, below the reference value for healthy individuals (70-110µg/dL). With the average concentration of the erythrocyte, it was noted values above (61.4 ± 15.6 µg/gHb) the established reference range (40-44µg/gHb). The enzymatic activity of SOD showed a great variability among patients, with a mean (1503.5 ± 581.2 U/gHb) within the reference range (1102-1601 U/gHb). The results of GPx showed a low enzyme activity (18.6 ± 6.0 U/gHb) when compared with reference values (27.5-73.6U/gHb). With the results obtained so far, controversial values for zinc concentrations as well as for the activity of antioxidant enzymes were observed, suggesting that a larger number of patients should be evaluated to obtain more conclusive results.

NUTRITIONAL SELENIUM STATUS AND ITS RELEVANCE TO GLUTATIONE PEROXIDASE ACTIVITY IN PATIENTS WITH RHEUMATOID ARTHRITIS

*Katia Rau De Almeida Callou, Graziela Biude Silva, Daniel Feldman Pollak, José Alexandre Pimentel, Sílvia M. Franciscato Cozzolino.

University of São Paulo. Department of Food Scince and Experimental Nutrition, Nutrition and Minerals Laboratory

Introduction - Selenium is an essential trace element associated with the function of major metabolic pathways in the cell. It is present in catalytic sites of the most important antioxidant enzyme glutathione peroxidase (GPX), protecting cells from oxidant damage. Impaired selenium status have been implicated in pathogenetic events in rheumatoid arthritis (RA) and could be a consequence of deficient selenium intake or as a result of inflammatory aspects of the disease. However, data envolving these aspects are scarce in literature. Objectives – The present study aimed to evaluate nutritional selenium status and their association with selenium intake and total GPX activity in RA patients. Sixteen RA patients aged 30-65 y and attending clinical treatment in São Paulo Hospital were included in this study. Besides that, inclusion criteria were the absence of other diseases and the absence of intake of medicine that could interfere with selenium biochemical profile. Methods -Blood samples were measured for selenium concentration (plasma, erythrocyte) by hydride generation atomic absorption spectrometry and total GPX activity using Kit Randox. Results are expressed by means of media and standard deviation. Results - All patients presented erythrocyte and plasma selenium concentration in the range of 42.6 ± 1.4 µgSe/l and 65.36 ± 1.77 µgSe/l, respectively. Selenium intake of these patients was lower (33.6 ± 16.6 µgSe/day) than the Dietary reference Intake Recommendation (60 µgSe/day) and GPX activity was decreased in all patients evaluated (45.68 ± 21.67 U/gHb). Besides that, a positive and significant correlation was found between erythrocyte selenium and GPX activity ($r = 0.666$, $p<0.05$). Conclusion – Nutritional selenium status was decreased, such considering serum and erythrocyte parameters as its mineral intake. This parameter indicate that, probably, selenium status wasn't enough to maximize the GPX activity in rheumatoid arthritis patients.

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TRACE ELEMENTS, OXIDATIVE STRESS AND GLYCEMIC CONTROL IN YOUNG PEOPLE WITH TYPE 1 DIABETES MELLITUS

Ching-Chiang Lin[a], Hsiu-Hua Huang[b], Yeou-Lih Huang[c]

a Department of Laboratory Medicine, Fooyin University Hospital, Pingtung, Taiwan
b Department of Laboratory Medicine, Kaohsiung Municipal Hsiaokang Hospital, Kaohsiung, Taiwan
c Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University, Kaohsiung, Taiwan (e-mail: yelihu@kmu.edu.tw)

In this study we analyzed trace elements and several oxidative parameters in patients with type 1 diabetes mellitus—including the levels of copper and zinc, superoxide dismutase (SOD) activity, blood and urinary malondialdehyde (MDA), and 8-hydroxy-2′-deoxyguanosine (8-OHdG)—to evaluate the relationship between these oxidative markers and glycemic control. Diabetic patients, especially in poorly controlled subjects, exhibited significantly lower levels of zinc and increased levels of copper (and, therefore, copper-to-zinc ratio), serum SOD, blood MDA, urinary MDA, and 8-OHdG, relative to healthy subjects. Furthermore, significant correlations existed between the serum copper, copper-to-zinc ratio, urinary MDA, 8-OHdG, and HbA1c levels in these patients. Our results suggest a significant correlation between oxidative stress and glycemic control; therefore, strict glycemic control and decreased oxidative stress might prevent diabetic complications in patients with type 1 diabetes mellitus.

1-50

DECREASED GLUTATHIONE LEVELS ARE NOT RELATED TO ANTIPSYCHOTIC MEDICATION NEITHER TO EXTRA-PYRAMIDAL SYMPTOMS IN PATIENTS WITH SCHIZOPHRENIA

Monia Raffa 1, Ihab Belhaj Youssef 2, Fatma Atig 1, Mohamed Tahar Sfar 1, Anwar Mechri 2, Abdelhamid Kerkeni 1

1 Research Laboratory of "Trace elements, free radicals and antioxidants", Biophysical Department, Faculty of Medicine, University of Monastir, 5000 Monastir, Tunisia
2 Department of Psychiatry, University Hospital of Monastir, 5000 Monastir, Tunisia
e-mail address: raffa_monia@yahoo.com

Altered glutathione (GSH) systems are suggested to participate in the physiopathology of schizophrenia.

Objectives: The aims of this study were to determine plasma GSH levels in patients with schizophrenia compared to healthy controls and to examine the relationships between GSH levels and therapeutic features and extra pyramidal symptoms in a sample of schizophrenic patients.

Methods: A case-control study carried-out on 100 patients (80 men and 20 women, mean age =31.56±6.83 years) with schizophrenia according to DSM-IV criteria, and 95 healthy control subjects (77 men and 18 women mean age = 31.19±5.49 years). The most of patients (59.1%) were under first-generation antipsychotics with a mean daily dosage of 403.1 ± 205.9 mg Chlorpromazine equivalents. Extra pyramidal symptoms were assessed by the Simpson and Angus scale (SAS). Plasmatic glutathione levels: total glutathione (GSHt), reduced glutathione (GSHr), oxidized glutathione (GSSG) were determined by spectrophotometry. Results: GSHt and GSHr levels were significantly lower in schizophrenic patients than in controls with respectively 647 ± 198.63 µmol/l versus 745.19 ± 223.87 µmol/l (p=0.006) for GSHt and 623.66 ± 199.11 µmol/l versus 718.56 ± 234.06 µmol/l (p=0.003) for GSHr. According to therapeutic status, there were no significant differences concerning GSH levels. In addition, there was no correlation between GSH levels and daily dosage of neuroleptic treatment in medicated schizophrenic patients. There was also no correlation between glutathione levels and SAS scores in schizophrenic patients.

Conclusion: These findings confirm the GSH decrease in patients with schizophrenia and suggest that decrease is not related to neuroleptic treatment or neurological side effects. Thus, the GSH deficit seems to be implicated in physiopathology of schizophrenia, and not related to therapeutic features.

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ASSOCIATIONS OF BLOOD LEVELS OF LEAD (PB), CADMIUM (CD) AND METHYLMERCURY (MEHG) WITH CARDIOVASCULAR DISEASE IN DIABETIC PATIENTS

Gómez Hoyos E[1], González-Estecha M[2] , Bordiu Obanza E[1], Cuesta Hernández M[1], Perez Ferre N[1], Calle Pascual A[1]

[1] *Endocrinology Service.* [2] *Trace Element Unit. Laboratory Medicine Department. Hospital Clínico San Carlos. Madrid. Spain E-mail: emigomezhoyos@hotmail.com*

Background. Chronic exposure to Pb, Cd and MeHg can be associated with cardiovascular disease. This study aims to determine the associations of blood levels of Pb, Cd and MeHg with CVD in type 2 diabetic patients.

Methods. Observational retrospective study. Group A(GA): 102 diabetic patients with peripheral vascular disease. Group B(GB): 89 patients with new onset diabetes and without history of cardiovascular disease. Blood Cd and Pb concentrations were determined by electrothermal atomic absorption spectrometry. Blood Mercury (Hg) concentrations were determined by cold vapour. Data were analyzed with SPSS 15.0.

Results. The median age of the GA patients (73.5%men) was 69.5 [56.0-74.75] vs 68 [54.5-73.0] in the GB patients (42% men). The median blood concentrations in GA and GB respectively were: for Pb 3.8 [2.6- 5.4] µg/dl vs 3.2 [2.1- 4.4] µg/dl , for Cd 0.10 [0.07-0.5] µg/l vs 0.07 [0.07-0.3] µg/l and for Hg 9.1 µg/l [5.4 –15.12] vs 5.8 [3.7- 11.4] µg/l. We found statistically significant differences between groups in the blood concentrations of Hg ($p<0.01$) and Pb($p<0.03$).

The median value of the blood concentrations in both groups divided by sex was, for men of GA and GB respectively, Pb 4.1 [3.1- 5.7] µg/dl vs 3.1 [2.3- 4.3] µg/dl, Cd 0.20 [0.07-0.6] µg/l vs 0.10 [0,07-0,3] µg/l and Hg 9.7[5.7–15.5] µg/l vs 6.6 [4.5-12.0] µg/l and we found no differences. Among female patients the median blood concentrations in Group A and B respectively were Pb 3.0 [1.7- 3.9] µg/dl vs 2.6 [1.8- 4.9] µg/dl, Cd 0.07 [0.07-0.2] µg/l vs 0.07 [0.07-0.5] µg/l and Hg 8.5 [4.8 – 14.8] µg/l vs 5.3[3.3 – 9.2] µg/l also without differences between groups.

However we found that in GA patients the blood Pb concentration was significantly higher in men compared with women ($p<0.004$), and in GB patients, the Hg concentration was also higher in men than women ($p<0.015$). In the univariant analysis, the difference in the blood Hg concentrations was attributable to the effect of the group ($p<0.02$) and the one in the blood Pb concentrations could be attributable to the gender effect ($p<0.001$).

Conclusion. Diabetic patients with known CVD had significantly higher levels of MeHg and Pb than those with recently diagnosed diabetes and no CVD. After adjustment by gender we found no association between the levels of Pb and CVD.

1-52

BLOOD MERCURY AND SERUM SELENIUM AND THEIR RELATIONSHIP WITH ESTIMATED GLOMERULAR FILTRATION USING CKD-EPI EQUATION

Gómez Hoyos E*,González-Estecha M**, Castillo Pérez C**, Diaz-Díaz MA**, Abad-Cardiel M***, García-Donaire JA***, Martell Claros N***, Arroyo M**.

Endocrinology Department. Laboratory Medicine Department. Trace Element Unit. ***Hypertension Unit. Hospital Clínico San Carlos. Madrid (España)*
Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC). Madrid (Spain).
Email: montse@cmpx.net

Background. Blood mercury has been associated with nephrotoxicity and cardiovascular disease among the general population. The aim of this study is to evaluate the association between blood mercury concentrations and estimated glomerular filtration rate (eGFR) adjusted by associated factors in a hospital working population.
Material and Methods. We recruited 395 employees (64 men and 331 women) with a mean age of 47.3 (SD: 10.9) years old. Blood mercury concentrations (µg/L) were measured by atomic absorption spectrometry and thermal decomposition amalgamation. Serum selenium concentrations (µg/L) were measured by electrothermal atomic absorption spectrometry. eGFR was assessed using the CDK-EPI equation.
Results. The median of blood mercury was 8 µg/L (IQR:5.2-11.6) and the mean of eGFR was 78.9 mL/min/l.73 m² (SD:11.9). The mean of serum selenium was 79.5 µg/L (SD: 11.9). Patients with an eGFR≤75 had a higher median of mercury (9 µg/L; IQR:6-13.6) than those with an eGFR>75 (7.3; IQR 4.9-10.5; p=0.004). We adjusted a logistic regression model in order to estimate the effect of mercury levels higher than 8 µg/L on the eGFR adjusting by age, glucose, hypertension, uric acid, LDL-C, lead, cadmium and selenium. We found a significant interaction with age (p<0.001). Mercury in subjects under 50 years old was associated in an independent way with the eGFR (OR 2.6 (95% CI: 1.3-5.2;p=0.005). We did not find this association in subjects over 50 years old (OR:1.03 (95% CI:0.54-1.98;p=0.930).
Conclusions. We found an association between blood mercury and eGFR in participants under 50 years old. The high blood mercury concentrations observed in this study as well as the association with a low eGFR support the need to perform more studies in the Spanish adult population in order to establish fish consumption recommendations.

1-53

ANTI-INFLAMMATORY AND ANTI-ULCEROGENIC ACTIVITY OF ZINC-XANTHONE COMPLEXES IN RATS

Anna Lipkowska 1, Magdalena Gawel 1, Tadeusz Librowski1, Gabriel Nowak1.3, Henryk Marona2, Natalia Szkaradek2

1 Department of Radioligands Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, 2Department of Drug's Chemistry Jagiellonian University Medical College, 3Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland, lipkowska.anna@gmail.com

Zinc is one of the most important trace element for living organisms. It acts in numerous processes such like proper functioning of enzymes, cell proliferation and differentiation. Studies have revealed that supplementation of zinc improves the anti-inflammatory of NSAIDS and protect gastric mucosa against ulceration at the same time. Even some compounds of zinc have anti-edematous activity. Previous researches have shown that xanthone derivatives besides other properties also act as an anti-inflammatory agents. The aim of this study was to evaluate how complexation with zinc influence on anti-inflammatory activity of xanthone derivatives does. The experiments were carried out on rats, male albino Wistar (120-250g). The complex was given per os. The first phase of inflammation was examined in formaline test. Anti-edematous activity was determined using carrageenan-induced hind paw edema test developed by Winter et al. The irritant action on the gastric mucosa was assessed macroscopically in accordance to Komatsu. The results show that the complexes have stronger anti-inflammatory activity than xanthone. The first phase of inflammation was effectively inhibited and the percent of edema reduction was significantly higher when the complex was used. Complexation with zinc enhanced the anti-edematous and analgesic activity of xanthone in all experiments. In future more research will be conducted to further explore complexes.

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INDAPAMIDE DECREASES ZINC AND COPPER STATUS, AND ALSO DISTURBS GLUCOSE CONCENTRATION AND PANCREATIC MASS, IN SHR RATS

J Suliburska1, Y Bogdanski2

1 *Department of Hygiene and Human Nutrition, Poznan University of Life Sciences, Poznan, Poland*
2 *Department of Internal Medicine, Metabolic Disorders and Hypertension, Poznan University of Medical Sciences, Poznan, Poland*
Corresponding author: Joanna Suliburska, Pharm.D., Department of Hygiene and Human Nutrition, Poznan University of Life Sciences, Wojska Polskiego 31 Str., 60-624 Poznan, Poland; Tel: 48(61)8487334, Fax: 48(61)8487332 jsulibur@up.poznan.pl

Mineral status in hypertensive patients may be affected by hypotensive drugs. Hypotensive drugs may also influence carbohydrate and lipid metabolism. The aim of this study was to assess the influence of indapamide on mineral status and selected biochemical and nutritional parameters. The experiment was performed with the agreement of the local bioethics committee (approval no. 49/2009).

The experiment was performed using 20 eight-week-old male spontaneously hypertensive rats (SHR). The animals were randomly assigned to two groups, equal in size: control and study. The SHR rats were fed a standard diet (maintenance diet for rats 1320, Altromin). Indapamide (6 mg/kg) was added to the diet of the study group. Ali rats were provided ad libitum diet and distilled water for 45 days. At the end of the experimental period, the animals were weighed, anesthetized, and killed by cardiac puncture. The liver, kidney, spleen, pancreas and heart were dissected, weighed, and stored frozen for analysis of mineral contents. The blood samples were collected and the serum and erythrocytes were separated and stored until analysis. Biochemical and morphological parameters were assayed in a diagnostic laboratory using routine methods. Blood pressure was measured with a tail-cuff plethysmograph, using a blood-pressure measuring system (MODEl MK-1030, Muromachi Kikai).

The iron, zinc, and copper contents in the tissues were determined after digestion, and the concentration of minerals was measured using the flame atomic absorption spectrometry method (AAS-3, Carl Zeiss, Jena, Germany).

Detailed statistical analysis was performed using Statistica for Windows 10.0 (StatSoft, Inc., Poland). The T-test was used to compare data between groups. Significance was set at the $P < 0.05$ level.

The average daily intake of the diet was comparable between the groups. The use of indapamide led to a significant decrease in the systolic and diastolic blood pressure in rats. The relative mass of the pancreas was markedly lower in the study group. It was found that indapamide led to a significant increase in the serum glucose concentration. The counts of white and red blood cells, as well as the hematocrit level, were higher in

the blood of rats with indapamide. It was also shown that indapamide significantly decreases zinc and copper levels in the liver and erythrocyte and copper levels in the kidneys in SHR rats.

Indapamide leads to a decrease in the zinc and copper status and also reduces the mass of the pancreas, conditions that may be associated with disorders of carbohydrate metabolism.

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1-55

ANALYSIS OF TRACE ELEMENTS IN HAIR AT PATIENTS WITH CAROTID ATHEROSCLEROSIS

Rozikhodjaeva G.A., Yokubov K. R., Hikmatov S.K., Hudoynazarov S.K., Shukurov A.N , Matchanov A.D

[1]Central Clinical Hospital #1 of Medicosanitary Associatio
[2] Republican Pathoanatomical Center, [3] Sanitary-epidemiologic station of Medicosanitary Association, Uzbekistan
e-mail: gulnoradm@inbox.ru

The purpose was studying of trace element levels (TE) (Fe, Cu, Al, Zn, Cd, Se, Ca, K, Na, Mg), and relationship between the levels of TE (Cu / Fe, Mg / Fe, Zn / Fe, Mg / Zn, Ca / Mg, K / Na, Zn / Cu, Cu / Zn) in hair of patients with carotid atherosclerosis (CA) compared with healthy people

Materials and methods. 27 men with CA and 10 healthy men are included in the study. The main and the control group were matched for age (mean age 61,2 ± 6,7 years and 64,4 ±9,6 years, respectively). Complex vascular examination was conducted on ultrasound scanners HD3 (Phillips, Holland). The intima – media thickness of common carotid artery (CIMT ACC) is measured at duplex scanning. TE in the hair was determined by optical emission spectrometry with inductively coupled argon plasma (OES-ICP) (Optima-2400DV (USA).

Results. Comparative analysis of TE composition of hair in patients with CA and healthy subjects showed an increased content of iron (Fe), aluminum (Al) in their hair compared with healthy, respectively, by 12%, 33% and a decrease in concentration of magnesium, zinc and copper by 15%, 8% and 36%, respectively. The study shows a negative correlation between Mg and Al. In patients with CA revealed significant positive correlation between the content of Mg and Fe, Cu and Al, Cu, Cd, Fe and Al.

Conclusion. The identified relationship between the content of TE in hair suggest the possibility of determining these parameters in patients as additional diagnostic criteria for complex examination.

1-56

ZINC AND SELENIUM SUPPLEMENTATION IN ATHEROSCLEROSIS PATIENTS TREATED WITH ROSUVASTATIN: A DOUBLE-BLIND RANDOMIZED CONTROLLED STUDY

Karine CM Sena-Evangelista[1], Lucia FC Pedrosa[1*], Maria SMO Paiva[2], Paula CS Dias[3], Diana QC Ferreira[3], Sílvia MF Cozzolino[4],Tanize ES Faulin[5], Dulcineia SP Abdalla[5]

[1]*Department of Nutrition, Federal University of Rio Grande do Norte, Brazil;*
[2] *Onofre Lopes University Hospital, Federal University of Rio Grande do Norte, Brazil;*
[3]*Post-Graduate Program in Pharmaceutical Sciences, Federal University of Rio Grande do Norte, Brazil;*
[4]*Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, Brazil*
[5]*Department of Clinical and Toxicological Analyses, Faculty of Pharmaceutical Sciences, University of São Paulo, Brazil*
(*) Corresponding author: lpedrosa@ufrnet.br

Background and objective: Zinc and selenium are important minerals for maintaining endothelial integrity. Statin treatments may cause atherosclerosis patients to experience alterations in levels of these minerals. The aim of this study was compare the effects of rosuvastatin treatment alone, and a combination rosuvastatin/mineral supplement treatment, on atherosclerosis patients exhibiting stable angina.

Methods: We performed a double-blind randomized clinical trial in which patients (n = 76) were treated with 10 mg of rosuvastatin over 4 months and received either a zinc (30 mg/d) and selenium (150 µg/d) (n = 38) supplement, or a placebo (n = 38). The following parameters were analyzed both before and after the intervention: anthropometrics, zinc and selenium in plasma and erythrocytes, lipid profiles, LDL(-) concentrations, and erythrocyte activities of GPx and SOD.

Results: Rosuvastatin therapy was efficient in reducing total, LDL, and non-HDL cholesterol and triglycerides independently of mineral supplementation. Neither treatment was associated with significant differences in concentrations of zinc (plasma: $p>0.14$; erythrocyte: $p>0.14$) or selenium (plasma: $p>0.63$; erythrocyte: $p>0.07$). Neither treatment regime was associated with significant changes in LDL(-) ($p>0.16$). Similarly, the antioxidant enzymes GPx and SOD were unchanged by either treatment regime (all $p>0.42$) and were similar in both groups (both $p>0.33$). However, the mineral-supplemented group had slightly higher values of both enzymes prior to the treatment. Neither treatment impacted zinc and selenium statuses or antioxidant activity.

Conclusion: Treatment with rosuvastatin did not have significant impacts on zinc and selenium status or on any of the mineral-associated biomarkers assessed in this study. Further, the zinc and selenium doses used here did not act synergistically or additively with rosuvastatin to alter the antioxidant statuses.

Trial was registered as NCT01547377.Clinicaltrials.gov.

1-57

SERUM SELENIUM AND THYROID FUNCTION INDICES IN FEMALE PATIENTS WITH HASHIMOTO DISEASE – PILOT STUDY

Jadwiga Kryczyk[1], Anna Blażewska-Gruszczyk[2], Marian Slowiaczek[2], Pawel Zagrodzki[1,3]

[1)] Dept. of Food Chemistry and Nutrition, Medical College, Jagiellonian University, Kraków, Poland, jadwiga.kryczyk@gmail.com;
[2)] Outpatient Clinic NZOZ DIAGNOZA; Kraków, Poland, annbl@esculap.pl, mariansl@esculap.pl; [3)] H. Niewodniczański Institute of Nuclear Physic, Dept. of Nuclear Physical Chemistry, Kraków, Poland, Pawel.Zagrodzki@ifj.edu.pl

The spectrum of interactions between selenium and the thyroid hormone axis is very broad, including the enzymes involved in the conversion of thyroid hormones (iodothyronine deiodinases) and two other families of selenoenzymes (glutathione peroxidases and thioredoxin reductases) which protect the thyrocytes from oxidative stress. Recently, it was found that Se supplementation inhibits production of anti-TPO in patients with Hashimoto thyroiditis (HT). HT is chronic disease often leading to hypothyroidism. The mechanism of action of selenium in patients with HT is unknown.

Therefore, this study aimed to clarify the role of selenium in euthyroid women with recently diagnosed and previously untreated Hashimoto disease. The set of parameters comprised serum selenium level (GF-AAS), glutathione peroxidase (Paglia & Valentine method), and serum concentrations of thyroid function indices: TSH, fT_4, T_3 and anti-TPO (routine methods). We investigated 9 women (36.4±8.1 years) with HT with normal values of TSH, and age- and sex-matched 9 healthy controls (38.8±5.5 years). The exclusion criteria were (1) current hormonal treatment, use of hormonal drugs or oral contraceptives, (2) taking any drugs known to influence basal serum levels of the hormones investigated, (3) a history of prior thyroid surgery, (4) non-regular menstrual cycle, (5) smoking, (6) dieting, (7) alcohol abuse, (8) pregnancy, (9) intensive sport exercising.

There was no significant difference in selenium concentration between HT patients (0.91±0.15 μM/L) and controls (0.81±0.12 μM/L). Statistically significant Partial Least Square model, which correlated the predictive parameters (TSH, fT_4, T_3 and Se) and response one (anti-TPO), revealed many significant correlations between hormones as well as positive correlations between Se and TSH, Se and fT_4 and negative correlation between Se and T_3. These findings support the concept that selenium status and thyroid physiology are interrelated in patients with HT.

1-58

THE RELATIONSHIP BETWEEN RESPIRATORY DISEASES AND ELEMENT STATUS OF POPULATION IN EUROPEAN PART OF RUSSIA

E.S. Berezkina (Vyatchanina)[1,2], A.R. Grabeklis[1,2], V.A. Demidov[3], V.Yu. Detkov[4]

[1] *Institute of toxicology FMBA, Saint Petersburg, Russia*
[2] *Russian Society of Trace Elements in Medicine, Moscow, Russia*
[3] *ANO Center of Biotic Medicine, Moscow, Russia*
[4] *Orenburg State University, Orenburg, Russia*
E-mail: aposteriori2@gmail.com

The relationship between element status of people and prevalence of respiratory diseases in them was studied. Hair samples from 18866 people (adults and children from North-European, South-European and Caucasian parts of Russia) were subjected to multielement analysis by ICP-AES/ICP-MS, and the obtained data were compared with official statistics on the morbidity for 2005-2006. It was shown that prevalence of respiratory diseases in adults correlated with increased incidence of Hg excess ($r=0.47$, $p<0.05$), Na deficiency (positively - $r=0.52$, $p<0.05$) and Mn deficiency (negatively - $r=-0.48$, $p<0.05$); with reduced incidence of K ($r=-0.56$, $p<0.02$), Na ($r=-0.57$, $p<0.02$) and P excess ($r=-0.49$, $p<0.05$). In children, the prevalence of respiratory diseases is growing with increased proportion of individuals with low Ca ($r=0.68$, $p<0.01$), Na ($r=0.69$, $p<0.01$), P ($r=0.63$, $p<0.01$) and Si ($r=0.72$, $p<0.01$). The fewer children have normal levels of hair Ca, P, Cu, Al, the higher the prevalence of this diseases (respectively, $r=-0.47$, -0.63, -0.65, -0.63, $p<0.05$). Thus, K/Na imbalance on the background of excessive accumulation of Hg may increase the prevalence of respiratory diseases in adults, and in children the leading role may belong to disturbances in exchange of Ca, P, Na and trace elements Cu and Si. Also the cases of bronchial asthma as a bright ecologically dependent disease were particularly studied. According to the correlation analysis, the increased prevalence of asthma in adults was connected with excessive accumulation of Hg ($r=0.52$, $p<0.05$), deficient Ca ($r=0.75$, $p<0.001$), Mg ($r=0.75$, $p<0,001$), K ($r=0.60$, $p<0.01$), Na ($r=0.49$, $p<0.05$), high Fe ($r=0.64$, $p<0.01$) and Mn ($r=0.49$, $p<0.05$). The more people had normal levels of Si, Ca, Na, Hg, the lower was the prevalence of asthma in adults (respectively, $r=-0.54$, -0.68, -0.50, -0.52, $p<0.05$). In children asthma was associated with Ca deficiency ($r=0.69$, $p<0.01$), abnormal levels of Cu and Al ($r=-0.56$, -0.63, $p<0.02$).

THE COPPER AND ZINC TISSUE CONCENTRATION IN PELVIC ORGAN PROLAPSE FORMATION IN WOMAN- PRELIMINARY STUDY

Marta Balajewicz-Nowak1 , Agnieszka Wojtanowska-Krośniak2, Miroslaw Krośniak2, Kazimierz Pityński1

1) Department of Gynecology and Obstetrics, Medical College, Jagiellonian University, Cracow, Poland mbalajewicz@cm-uj.krakow.pl,

2) Department of Food Chemistry and Nutrittion, Medical College, Jagiellonian University, Cracow, Poland a.wojtanowska-krosniak@uj.edu.pl, mfkrosni@cyf-kr.edu.pl

Pelvic Organ Prolapse (POP) is a common group of clinical conditions affecting increasing group of women in our population. The risk factors of POP consist of age, obesity, parity, chronic cough, chronic constipation, heavy lifting, surgery. Women with POP often have urinary incontinence, which frequently is the first symptom reported by patients. The lifetime risk of developing POP is about 14% and its increasing, about 50% of patients will need a surgery and up to one third will require a repeated procedure. The cost effectiveness of surgical treatment, reconvalescence, rehabilitation and follow-up are very high, also the psychological and sociological effects are as important as economical.

An active investigation of new pathophysiological factors initiating or promoting POP, which in future could be the foundation to create POP prophylactic programs or new methods of treatment. Lysyl oxidase (protein-lysine 6-oxidase, LOX) is a copper-dependent enzyme. Our aim was to determine the correlation between copper and zinc plasma concentration in tissue pelvic support system in women suffering from POP. Additionally our aim was to asses microelements status in pelvic support system in women suffering from POP. 31 patients were evaluated and divided into two groups. First group – study population: woman with pelvic organ prolapse and the control population: women operated due to uterine myoma, who agreed to take part in research. During standard surgical procedure tissue of pelvic support system was collected and storaged. Tissues after lyophilization and digestion were studied using flame AAS method. Concentration of analyzed elements: Mg, , Ca, Cu and Zn level were calculated for dry mass of tissues. Statistic analysis were calculated using Statistica 10 software and ANOVA Kruskal-Walis test and Spearman correlation

Mean concentration of Mg was 301.8 $\pm$ 87.8 mg/kg, for Ca was 604.3 $\pm$ 164.5 mg/kg, for Cu was 4.741 $\pm$ 1.69 mg/kg and for Zn was 69.91 $\pm$ 15.37 mg/kg. Our study showed lower concentration of Zn level in group of women with pelvic organ prolapse in comparison to control group ($p<0,05$). Copper dry tissue concentration did not differ between the groups.

The microelements may take part in POP formation, but further study are necessary to clear this issue.

1-60

THE INFLUENCE OF ZINC IONS ON THE ANTI-INFLAMMATORY AND ULCEROGENIC ACTIVITY OF KETOPROFEN

Gawel Magdalena, Lipkowska Anna, Librowski Tadeusz

Department of Radioligands, Jagiellonian University, Medical College, Medyczna 9, 30-688 Kraków, Poland
magdalena.gawel@uj.edu.pl

Zinc is an essential trace element required for the proper functioning of numerous proteins, cell proliferation and differentiation. Studies have demonstrated that zinc acts as an antioxidant and anti-inflammatory agent protecting mucosal cells against induced gastric ulcers in animal models. Non-steroidal anti-inflammatory drugs, though commonly used and usually well tolerated by patients, frequently cause gastrointestinal erosions and ulcerations. The study's aim was to evaluate the influence of single and multiple administration (p.o. and i.p.) of selected zinc compounds on the anti-inflammatory and ulcerogenic activity of ketoprofen. The experiments were carried out on rats, male albino Wistar (140-290 g). Anti-edematous activity was determined using carrageenan-induced hind paw edema test developed by Winter et al. The irritant action on the gastric mucosa was assessed macroscopically in accordance to Komatsu. The results show that the percent of edema reduction in rats depends on the doses used, duration of the experiment and route of administration. Zinc enhanced the anti-edematous activity of ketoprofen in all experiments. Some of the selected zinc compounds given alone without ketoprofen caused the significant reduction of the edema in comparison to the control groups. It was assessed that zinc alleviated gastrointestinal injuries caused by ketoprofen, however more research are being conducted to confirm the gastroprotective properties of selected zinc compounds.

9. Inherited disorders

1-61

COPPER AND ZINC IN THE SERUM, URINE, AND HAIR OF PATIENTS WITH WILSON'S DISEASE TREATED WITH PENICILLAMINE AND ZINC

Dastych M [1], Prochazkova D [2]

[1]*Dep. of Clinical Biochemistry University Hospital Brno and Dep. of Laboratory Methods Medical Faculty Masaryk University, Czech Republic, mdast@fnbrno.cz*
[2] *Medical Faculty Masaryk University, Czech Republic,prochazkova@fnbrno.cz*

Introduction. The purpose of this study was to determine the different levels of copper and zinc in the serum, urine, and scalp hair of patients with Wilson's disease receiving different, currently accepted methods of treatment to reduce the copper load

Patients. Penicillamine-group 1, n=8; zinc-group 2, n=8; penicillamine+zinc-group 3, n=8). Blood, urine, and hair samples were collected from the patients.

Results.All three treatments resulted in a significant decrease of the serum copper levels. Significantly increased levels of zinc in the serum were detected in the patients in groups 2 and 3 (19.1 and 18.8 μmol/l, respectively; $p<0.05$). Copper excretion in the urine significantly increased during its administration to groups 1 and 3 (11.5 and 7.94 μmol/24 h respectively; $p<0.001$) due to the effect of penicillamine. The administration of zinc as monotherapy (group 2) or in combination with penicillamine (group 3) led to an increase of its excretion (25.3 and 22.4 μmol/24 h, respectively; $p<0.01$). Only an insignificant rise of the copper content in the hair was found in all three groups of patients. The content of zinc in the hair did not differ significantly in any of the groups in comparison with the control group.

Discussion. Penicillamine is the treatment of choice in treating adult patients in the Czech Republic to this day. Using zinc in monotherapy or in combination with penicillamine is less frequent. Combined therapy and zinc monotherapy is much more frequent in pediatric praxis.

men, from 23 to 63 years of age (mean 44 years) were included. Nine

were C282Y homozygote, one was C282Y heterozygote, three were H63D homozygote, five were compound heterozygote and two had none of the mutations above. Iron and liver function tests were performed; serum level of the elements B, Ba, Co, Cs, Cu, Mn, Mo, Ni, Se, Sr, Y and Zn were measured using inductively coupled plasma mass spectrometry.

Results before the start of treatment and after normalization of iron parameters were compared.

Results : Serum ferritin before the start of treatment ranged from 249 – 2555 µg/L (mean 764 µg/L) and fell to 18-140 µg/L (mean 63 µg/L) after treatment. On completion of the bloodlettings the serum concentration of Co had increased in all patients (from average 5,5 to 12,4 nmol/L), serum Cu increased in 19 out of 20 patients (from average 15,8 to 17,5 µmol/L) 9and serum Ni increased in 16 of the patients (from average 51 to 54 nmol/L). Serum Mn declined in 16 of the patients (from average 32 to 29 nmol/L). All changes were statistically significant (by paired t-test). Serum levels of Zn, Se, Y, B, Sr, Mo, Cs and Ba were not significantly changed.

Conclusion : Bloodletting for hemochromatosis does affect the homeostasis of some trace elements

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BLOODLETTING THERAPY IN HEMOCHROMATOSIS: DOES IT AFFECT TRACE ELEMENT HOMEOSTASIS?

Bjørn J. Bolann[1,3], Sonia Distante[2], Lars Mørkrid[2] and Rune J. Ulvik[1]

[1]*Institute of Medicine, University of Bergen and Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen*
[2]*Department of Medical Biochemistry, Oslo University Hospital, Norway. bjorn.bolann@med.uib.no*

Hemochromatosis is the most common hereditary disorder in the Nordic population, if left untreated it can result in severe parenchymal iron accumulation. Bloodletting is mainstay treatment; 450-500 mL of blood is withdrawn weekly, and patients can undergo up to 20-40 bloodlettings to achieve normalization of iron parameters. Iron and trace elements partially share cellular uptake and transport mechanisms, and the aim of the present study was to see if bloodletting for hemochromatosis affects trace elements homeostasis.

Methods : We recruited patients referred to our clinics for diagnosis and treatment of hemochromatosis. Two women and 18 men, from 23 to 63 years of age (mean 44 years) were included. Nine were C282Y homozygote, one was C282Y heterozygote, three were H63D homozygote, five were compound heterozygote and two had none of the mutations above. Iron and liver function tests were performed; serum level of the elements B, Ba, Co, Cs, Cu, Mn, Mo, Ni, Se, Sr, Y and Zn were measured using inductively coupled plasma mass spectrometry.

Results before the start of treatment and after normalization of iron parameters were compared.

Results : Serum ferritin before the start of treatment ranged from 249 – 2555 µg/L (mean 764 µg/L) and fell to 18-140 µg/L (mean 63 µg/L) after treatment. On completion of the bloodlettings the serum concentration of Co had increased in all patients (from average 5,5 to 12,4 nmol/L), serum Cu increased in 19 out of 20 patients (from average 15,8 to 17,5 µmol/L) 9and serum Ni increased in 16 of the patients (from average 51 to 54 nmol/L). Serum Mn declined in 16 of the patients (from average 32 to 29 nmol/L). All changes were statistically significant (by paired t-test). Serum levels of Zn, Se, Y, B, Sr, Mo, Cs and Ba were not significantly changed.

Conclusion : Bloodletting for hemochromatosis does affect the homeostasis of some trace elements

1-63

BLOOD MERCURY AND SERUM SELENIUM IN A CYSTIC FIBROSIS POPULATION

Castillo Pérez C*, Diaz-Diaz A*, Ruiz L**, Prados C*** ,Arroyo M*, González-Estecha M*, Segovia J**.

**Laboratory Medicine Department. Trace Element Unit. Hospital Clínico San Carlos. Madrid (Spain)*
***Hospital Puerta de Hierro Majadahonda, Cardiology, Madrid, Spain*
**** Hospital Universitario La Paz, Neumology, Madrid, Spain*
Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC). Madrid (Spain).
Email: montse@cmpx.net

Background. Cystic fibrosis is characterized by an obstructive pulmonary pattern and a pancreatic exocrine deficiency frequently associated with malabsorption and prolonged antibiotic treatment. Certain studies have described a rare form of cardiomyopathy, similar to the one seen in Keshan's disease associated with a selenium deficiency. Exposure to mercury is a public health issue. The aim of this study is to analyze the concentration of blood mercury and serum selenium in patients with cystic fibrosis and the association between these elements.

Material and Methods. We studied 123 adult CF patients (63 male and 60 female) with a mean age of 31 (SD: 8.90). Blood mercury concentration (µg/L) was measured by atomic absorption spectrometry and thermal decomposition amalgamation. Serum selenium concentration (µg/L) was measured by electrothermal atomic absorption spectrometry.

Results. The median of blood mercury was 5.7 (IQR: 3-9.7) and the mean serum selenium was 71.9 (SD:14.8). We found a positive significant correlation between mercury and selenium ($r=0.308$;$p<0.001$). Considering the selenium deficiency, we also observed this correlation ($r=0.619$;$p=0.003$) in patients with a selenium concentration below 60 µg/L (N=22). However, we did not find this correlation ($r=0.096$,$p=0.11$) in the patients (N=110) with a selenium higher than 60 µg/L.

Conclusions. We found low selenium and high mercury concentrations in cystic fibrosis patients. The lack of association between these two elements in the group of patients with higher selenium concentrations suggests that certain factors such as malabsorption and antibiotic treatment can modify the absorption and metabolism of these trace elements.

10. Intensive care

BARIUM IN INTRAVENOUS SOLUTIONS FOR ADMINISTRATION TO NEONATES: ORIGINS AND LEVELS OF CONTAMINATION

Vanessa Morschbacher[a], Carine Viana Silva [b] and Denise Bohrer [a]

[a] *Department of Chemistry*
[b] *Department of Pharmaceutical Sciences, Federal University of Santa Maria (UFSM), Santa Maria-RS, Brazil. ndenise@quimica.ufsm.br*

Barium is a glass constituent and a component of plastic additives. Barium (Ba) may migrate from containers and devices into solutions and reach toxic levels. In this study, all steps involved in the preparation of intravenous solutions for premature neonates in an intensive care unit were evaluated to determine to what degree, if any, they contribute to Ba load. Commercial solutions for parenteral nutrition (PN) and medications as well as the apparatus used for administration were analyzed for their Ba content. Bags after compounding and medications after their preparation, as well as infusion sets and syringes, were also evaluated. Barium was determined by atomic absorption spectrometry. Bags, burettes, syringes, rubber caps, and glass containers showed Ba in levels varying from 0.02 to 4.38 mg Ba/g. Among the solutions, the highest levels were found in multivitamins, magnesium sulfate, and calcium gluconate, 262, 193, and 166 µg/L, respectively. Most medications did not present Ba contamination, however, after dilution in syringes, all of them became contaminated, being the highest level reached in dexamethasone samples, 1333 µg/L. Compounded PN bags (n=15) presented a mean of 54.6 µg/L Ba. The content of the same bags but after percolating the burette presented a mean level of 94.2 µg/L Ba. The handling process of compounding and delivering solutions and medicine increased the Ba intake by almost 3 times in relation to its level in the starting products. Considering the fragile conditions of premature neonates, the intake of a toxic species such as Ba should be of concern.

TRACE ELEMENT CONCENTRATIONS AND SEVERE INFLAMMATION

Sergey Sviridov[1], Mikhail Bolshov[2], Irina Seregina[2], Sergey Fedorov[1], Ruben Yagubyan[1], Konstantin Ossipov[2], Vladimir Kochergin[1].

1Department of Anesthesilogy and Intensive Care, The Russian National Research Medical University named after N.I. Pirogov, 2Analytical Chemistry Division, Lomonosov Moscow State University

Introduction. Studying the trace element status of patients with various inflammatory processes is becoming widespread in medicine nowadays. Big role in antioxidant defense have minerals zinc (Zn) and copper (Cu).

Aim: The aim of the study was to identify changes in concentration of Zn and Cu in whole blood and urine in patients with inflammatory process.

Materials and methods. The study included 20 patients (age 40.1 y0, SD - 33.9) with purulent wounds of soft tissues (PWST) or acute necrotizing pancreatitis (ANP), or peritonitis of any cause (P). They all were admitted to the intensive care unit (ICU) in severe or critical condition. Whole blood and urine of all patients in the first day after admission were analyzed for Zn and Cu concentration. All patients were followed to their discharge from the department or death.

Results. We found following mean concentrations of the trace elements: in whole blood: Zn concentration - 4267 µg/L, SD - 1131 µg/L, Cu - 747 µg/L, SD - 224 µg/L. The mean Zn concentration in the urine was – 1834 µg/L, SD - 1778 µg/L and Cu - 84 µg/L, SD - 99 µg/L. We found relation between reduced Cu concentrations in whole blood and the severity of disease (worst – P, less severe – ANP, the least severer – PWST) ($r = 0,6$, $p < 0,01$). Also the correlation between Cu concentration in urine greater than 70 µg/L and patient survival was significant and negative ($r = -0,9$, $p < 0,01$).

Conclusion. The severity of the inflammatory process has a direct impact on reducing Cu concentration in whole blood and increasing its excretion to the urine. The increase of Cu concentration in the urine is an independent worsening predictor of prognosis in patients with severe inflammation.

THE ALTERATIONS IN SELENOENZYME ACTIVITIES AND ANTIOXIDANT/OXIDANT PARAMETERS IN PROVEN NEONATAL SEPSIS

Ali Asci[1], Ozge Surmeli Onay[2], Pınar Erkekoglu[1], Murat Yurdakok[2], Sule Yigit[2], Belma Kocer Giray[1]

[1] *Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara/Turkey*
[2] *Hacettepe University, İhsan Doğramacı Childrens' Hospital, Neonatology Department, Ankara/Turkey*

Sepsis is caused by the immune system's response to a serious infection. When the infection cause is clarified by culture or stain, this condition is called "proven sepsis". Proven neonatal sepsis specifically refers to the presence of a bacterial blood stream infection and is the main cause of mortality in neonatal intensive care units. Selenium is essential for the proper functioning of immune system. Both selenoenzymes and selenoprotein P are shown to be important biomarkers in adult sepsis. However, there are few studies determining the alterations in the activities of selenoenzymes and the role of oxidative stress in proven neonatal sepsis. This study is designed to evaluate erythrocyte selenoenzyme activities (i.e. glutathione peroxidase [GPx1], thioredoxin reductase [TrxR]) and other oxidant/antioxidant parameters (i.e. activities of erthyrocyte catalase, superoxide dismutase; concentrations of erthyrocyte total glutathione [GSH]; plasma total antioxidant capacity, lipid peroxidation, and carbonyl levels) in proven neonatal sepsis (n=25) patients. In proven sepsis, marked decreases were observed both in TrxR activity (28%) and total GSH levels (35%) while no significant alteration was observed in GPx1 activity in comparison to controls (n=35). On the other hand, malondialdehyde levels increased significantly vs. control group. Significant correlations were observed between biomarkers of sepsis and oxidant/antioxidant status parameters in proven sepsis group [Ratio of immature/mature (IM/M) neutrophils–GPx1 activity ($r= 0.448$, $p<0.05$); IM/M neutrophils–MDA level ($r=0.417$, $p<0.05$)]. These results point out that at least one of the mechanisms underlying the molecular events observed in sepsis is oxidative stress and alterations in selenoenzyme activities can be important in the course of this disease.

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SODIUM SELENITE AS A THERAPEUTIC AGENT IN A LIPOPOLYSACCHARIDE RAT MODEL? ELEMENTS OF ACTION AND TOXICITY

Xavier Forceville [1], Samia Touati [1], Philippe Le Toumelin [2], Véronique Ducros [3], François Laporte [3], Yves Chancerelle [4], Diane Agay [4].

[1]. *Centre Hospitalier de Meaux, Réanimation Polyvalente, Meaux, France*
[2]. *Hôpital Avicenne, Bobigny Cedex, France*
[3]. *CHU de Grenoble, Département de Biochimie Toxicologie & Pharmacologie, Grenoble, France*
[4]. *Institut de Recherche Biomédicale des Armées, Département des Effets Biologiques des Rayonnements, La Tronche, France*

Purpose : Both septic shock and sodium selenite (Na_2SeO_3) lead to multiple organ failure through oxidation. Na_2SeO_3 has indirect anti-oxidant properties and direct oxidant effects above the nutritional level.
In a lipopolysaccharide (LPS) rat model we assessed beneficial effect and toxicity of pentahydrate Na_2SeO_3 ($5H_2O•Na_2SeO_3$) at oxidant doses.

Methods: In a three-step study on 204 rats we: (i) observed toxic effects of Na_2SeO_3 injected intraperitoneously (IP) and determined its Minimum Dose Without Toxic effect (MDWT) 0.25-0.35 mg/kg selenium (Se) content; (ii) injected IP LPS at 70% lethal dose (LD) followed, or not, one hour later by IP Na_2SeO_3 at MDWT and (iii) by doses > MDWT. At 48 hours, on survivors, we measured plasma creatinine, lactate, aspartate and alanine aminotransferase (AST, ALT), nitric oxide (NO) and Se concentrations.

Results : (i) Na_2SeO_3 alone did not increase NO and lactate. Encephalopathy appeared at 1 mg Se/kg. Creatinine increased at 1-1.75 mg Se/kg, AST, ALT at 3-4.5 mg Se/kg, and the minimum LD was 3 mg Se/kg. (ii) Mortality after LPS was 37/50 (74%, [62-86%]) vs. 20/30 (67%, [50-84%]) when followed by Na_2SeO_3 at MDWT ($p=0.483$) with a decreased in NO (-31%, $p=0.038$) a trend for lactate decrease (-19%, $p=0.068$) and an increased Se in survivals. (iii) All rats died at doses ≥0.6 mg/kg ($p<0.001$).

Conclusion: Mechanisms of LPS and Na_2SeO_3 toxicity differ (i.e. NO, lactate). In septic shock, IP administration of dose considered as oxidant of $5H_2O•Na_2SeO_3$ may be beneficial, but $5H_2O•Na_2SeO_3$ toxicity increased in septic shock.

11. Pediatrics

2-68

PRE- AND NEONATAL EXPOSURE OF RATS TO LEAD LEADING TO BLOOD PB CONCENTRATION BELOW THE THRESHOLD CONSIDERED SAFE FOR PEOPLE DECREASED THE LEVEL OF BDNF IN BRAIN

Baranowska-Bosiacka Irena1, Gutowska Izabela2, Piotrowska Katarzyna3, Rybicka Marta1, Dec Karolina2, Chlubek Dariusz1

1Department of Biochemistry, 2Department of Biochemistry and Human Nutrition, 3Department of Physiology, Pomeranian Medical University, Szczecin, Poland;
e-mail: ika@pum.edu.pl

Brain-derived neurotrophic factor (BDNF) plays important roles in the proliferation, differentiation and survival of neurons during development, as well as in the synaptic activity and plasticity of mature neurons, and is critically involved in synaptic transmission in the hippocampus, as well as in learning and memory. It has been shown that BDNF can also protect neurons from apoptosis.

The aim of this study was to examine if pre- and neonatal exposure to lead (Pb) may influence on BDNF concentration in the developing rat brain.

Pregnant experimental females received 0.1% lead acetate (PbAc) in drinking water from the first day of gestation until weaning of the offspring; the control group received distilled water. During the feeding of pups, mothers from the experimental group were still receiving PbAc. Pups were weaned at postnatal day 21 and the young rats of both groups then received only distilled water until postnatal day 28. This treatment protocol resulted in a concentration of Pb in rat offspring whole blood (Pb-B) below the threshold of 10 µg/dL, considered safe for humans. BDNF concentration was measured in forebrain cortex (FC), cerebellum (C) and hippocampus (H) of rats using the standard sandwich ELISA assay. In brain homogenates obtained from rats subjected to Pb-exposure, the concentration of BDNF was significantly lower in FC by 39% and in H by 29% in comparison to controls.

Pre- and neonatal exposure of rats to Pb, leading to Pb-B below 10 µg/dL (the threshold of Pb-B value considered safe for people) reduced level of BDNF in brain.

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NUTRITIONAL ANEMIA IN RURAL SCHOOLCHILDREN OF MOROCCO

S. Fadeli El, El Hari A, H. Benmazhar. N. Lekouch, A Sedki.

Laboratory of Ecotoxicology, Faculty of Sciences, Marrakesh, Morocco sedkiazeddine@gmail.com

Iron deficiency is a major nutritional problem in the world and particularly in Morocco. Anemia is a public health problem with major consequences extended both on the health of the economic, social and cognitive development of children in schools.

Objective: The aim of our study was to determine the prevalence of anemia and its association with socio-economic parameters and anthropometric school children in one of Marrakesh rural mining areas.

Methods: The study group was composed of 129 children aged between 3 to 9 years, coming from a rural origin with a modest socio-economic level (Drâa Lesfer Mining Area). Iron status was determined by hemoglobin, ferritin and serum iron. A questionnaire was developed to obtain information on the socio-economic and demographic family such as household size, income, possessions and parents' education level.

Results: The mean concentration of hemoglobin was 11.27 g / dl, while the average level of ferritin was 27 ng / ml and serum iron was 0.55 mg / l. The prevalence of anemia was 41%. Younger children are more vulnerable than their elders with mean hemoglobin of 10.8 g / dl for preschoolers against 11.79 g / dl for school children.

These alarming results needed to implement the development of an operational strategy with the main aim of fighting iron deficiency, based primarily on nutrition education.

2-70

LEAD SCREENING AROUND AN INDUSTRIAL SITE: EVALUATION OF CHILDHOOD LEAD POISONING

Sedki., S. El fadili, N. Lekouch. H. Benmazhar. H. Elhari

Cadi Ayyad University, Faculty of Sciences, LHEA Laboratory, Marrakesh
Sedki@uca.ma

Problematic

Children's exposure to lead, even at low doses, can cause neurobehavioral as well as intellectual ones. Studies on lead poisoning in children aged between 1 to 6 years and also in pregnant women showed that the industrial exhibition was a priority for implementation of screening for lead poisoning. Hence, it's important to define the selection criteria for the sites where a systematic screening should be done, since the measuring blood lead requires a blood sample, an invasive procedure or bioindicators of pollutant exposure such as appendages (baby teeth, hair and / or nails).

Methods

We propose to model the blood lead levels of children aged between 1 to 6 years, from environmental measurements, parameters of human exposure and the relationship between the dose of exposure and the blood lead. The Decision criteria proposed are, medium and unfavorable blood lead, predicted by the model. This method is illustrated by the case of a real lead-zinc mine in the region of Marrakesh.

Results

For the study area, the modeled exposures are less than 100 µg (Pb) / l(blood). Such dose do not lead to recommend a routine screening for childhood lead poisoning. However, these observed levels could cause a significant cognitive change in the children's behavior or even cause them the iron deficiency anemia.

Conclusion

The indirect estimation of the exposure associated with the blood lead prediction is useful in determining the relevance of childhood lead poisoning around industrial sites. Many ways to improve this decision support tool are proposed.

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SUSCEPTIBILITY OF CHILDREN TO EVIRONMENTAL XENOBIOTICS MEASURED BY CYTOKINESIS-BLOCK MN ASSAY

Lucyna Kapka-Skrzypczak1,2, Joanna Niedźwiecka1, Krzysztof Sawicki1, Malgorzata Cyranka1, Magdalena Wasak3, Waldemar A. Turski3, Marcin Kruszewski1,4

1Independent Laboratory of Molecular Biology, Institute of Rural Health, Lublin, Poland;
e-mail: lucynakapka@gmail.com
2Department of Public Health, University of Information Technology and Management, Rzeszów, Poland
3Department of Toxicology, Institute of Rural Health, Jaczewskiego 2, Lublin, Poland
4Centre for Radiobiology and Biological Dosimetry, Institute of Nuclear Chemistry and Technology, Warsaw, Poland

Certain classes of chemicals such as lead and pesticides have attracted attention from the scientific community, as well as from the media and the general public, for their potential impact on the health of children. Children have a greater vulnerability to the adverse health effects of many environmental toxicants and xenobiotics than do adults, by virtue of their smaller size, increased weight-adjusted intake of toxicants, developmental differences in body systems and functions, overall physiological and toxicodynamic differences, and differences in the environments they inhabit, the air they breathe, the foods they eat and the behaviours they display every day. The frequency of micronuclei (MN) in peripheral blood lymphocytes is extensively used as a biomarker of chromosomal damage and genome stability in human populations. We examined whether prolonged children`s environmental exposure to lead or pesticides leads to an increase in cytogenetic damage. The frequency of MN was determined by the cytokinesis-block MN assay. The examined population exposed to lead was composed of 9-year-old children (n=92), living in the region where non-ferrous ores are extracted and processed; the control group consisted of 49 children of the same age from an unexposed recreational area. The group exposed to pesticides comprised of 7-11-years-old children (n=100) living in agricultural area; the control group consisted of 88 childrea living in agritourism region, without indication of exposure to pesticides.The results for children exposed to lead showed a significant difference ($P < 0.0001$) in the level of MN between the exposed and control group (standard MN test: 2.96 +/- 2.36 versus 1.16 +/- 1.28). We also found that children exposed to pesticides, presented significantly higher MN frequency in lymphocytes than controls ($P<0.01$). Multiple regression analysis indicated that the exposure to lead or pesticides were the important factors affecting the increase in MN frequency. Our results show that children exposed to lead and pesticides might be more susceptible to DNA alterations.

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BLOOD MERCURY AND SERUM SELENIUM CONCENTRATIONS IN A PEDIATRIC POPULATION AND THEIR RELATIONSHIP WITH FISH CONSUMPTION

Gaviña B*, Bodas A**, Vieco A**, Blanco F***, Castillo Pérez C*, Diaz-Díaz MA*, Fuentes M*, González-Estecha M*.

**Laboratory Medicine Department. Trace Element Unit.*
***Pediatric Department. Hospital Clínico San Carlos. Madrid (Spain)*
****Neurology Department. Hospital de la Zarzuela.*
Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC). Madrid (Spain).
Email: montse@cmpx.net

Background. Methylmercury is a neurotoxic element that adversely affects neurocognitive development. Different countries have different recommendations in order to reduce the intake of methylmercury, especially in vulnerable groups such as children. The aim of this study is to measure the blood mercury and serum selenium concentrations in a child population and their association with fish consumption.

Material and Methods. We recruited 96 children (44 boys and 52 girls) with a mean age of 6.7 (SD:4.5) years old. Blood mercury concentrations (µg/L) were measured by atomic absorption spectrometry and thermal decomposition amalgamation. Serum selenium concentrations (µg/L) were measured by electrothermal atomic absorption spectrometry.

Results. A positive association was found (r=0.297;p<0.001) between blood mercury (median 2.2 IQR 1.1-4.5) and serum selenium concentrations (mean 69.5 SD:13.9). Children in the third tertile of mercury (>3.5 µg/L) had higher selenium concentrations (mean 64.98 vs 74.49;p=0.004) than children in the first tertile (≤1.35 µg/L). 18% of the children had a blood mercury concentration higher than the 5.8 µg/L limit established by the Environmental Protection Agency.

A statistically significant correlation was found between blood mercury concentrations and frequency of fish consumption: weekly (r=0.327;p<0.001), white fish (r=0.212;p=0.017), blue fish (r=0.344;p<0.001), swordfish (r=0.336;p<0.001) and tuna consumption (r=0.279;p=0.002).

Conclusions. The finding that a relatively high percentage of the children studied had blood mercury concentrations higher than the EPA recommendation supports the recommendation to limit the consumption of certain fish with high mercury content in Spain. More studies are needed to confirm the protective role of selenium given the association between serum selenium and blood mercury.

2-73

BLOOD LEAD AND CADMIUM LEVELS IN A PEDIATRIC POPULATION AND ITS RELATIONSHIP WITH RISK EXPOSURE FACTORS

Gaviña B*, Bodas A**, Blanco F***, Vieco A**, Castillo Pérez C*, Diaz-Díaz MA*, Ordóñez JM****, González-Estecha *M**.

**Laboratory Medicine Department. Trace Element Unit. Hospital Clínico San Carlos. Madrid (Spain)*
***Pedriatric Department. Hospital Clínico San Carlos. Madrid (Spain)*
****Neurology Department. Hospital de la Zarzuela.*
*****Consejería de Salud Pública. Comunidad de Madrid.*
Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC). Madrid (Spain).
Email: montse@cmpx.net

Background. Lead and cadmium exposure is a public health problem due to the widespread exposure and there is probably no safe threshold. The aim of this study is to measure blood lead and cadmium levels among the pediatric population that goes to routine medical check-ups and to identify associated factors.

Material and Methods. We recruited 85 children (42 boys and 43 girls) with a mean age of 10.5 (SD 2.3). Blood lead (µg/dL) and cadmium concentrations (µg/L) were measured by electrothermal atomic absorption spectrometry. A risk exposure questionnaire for lead and cadmium was administered to the participants.

Results. The median of blood lead was 1.1 (IQR 0.7-1.6 µg/dl). The mean of cadmium was 0.07 µg/l (SD: 0.05) and 94% of the samples were under the detection limit. We did not find significant differences in blood lead levels with respect to gender or nationality, nail-biting or sucking on objects, thumb-sucking, having pets, use of earthenware cooking vessels or the age of the residence. Children who played outdoors had higher blood lead levels than those who played at home (median 1.2 IQR 0.75-1.7 vs 0.9 IQR 0.40-1.4;p=0.025). Significant differences were also found when taking into account those parents who smoked (median 1.3 IQR 0.8-1.9 vs 0.80 IQR 0.52-1.3 of non- smokers; p=0.02). Children who drank tap water had higher blood lead levels (median 1.2 IQR 0.7-1.75) with respect to those who drank bottled waters (median 0.75 IQR 0.27-1.3) p=0.038. Children whose parents had not finished school had higher blood lead levels (median 1.85 IQR 0.7-2.32) than those whose parents had finished school (median 1.1 IQR 0.6-1.6) and those whose parents had gone to university (median 0.9 IQR 0.4-1.3) p=0.028).

Conclusions. In spite of the decline in blood levels, associated risk factors continue to exist in vulnerable populations such as children; thus, environmental control of this pollutant is indispensable.

2-74

INCENT BURNING AT HOME AND BLOOD LEAD LEVELS OF PRESCHOOLERS IN TAIWAN

Yaw-Huei Hwang[1,2], Yi-Hseun Lin[1], Chia-Yu Lin[1], Wang I-Jenc[3,4]

[1] *Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University, Rm 735, No.17, Xu-Zhou Rd., Taipei, 10055, Taiwan, R.O.C. e-mail: yawhueihwang@ntu.edu.tw*
[2] *Department of Public Health, College of Public Health, National Taiwan University, Taiwan, R.O.C.*
[3] *Department of Pediatrics, Taipei Hospital, Department of Health, Taipei, Taiwan, R.O.C.*
[4] *College of Public Health, China Medical University, Taichung, Taiwan, R.O.C.*

The growth and intelligent development of children less than six years old may be affected by low-level lead exposure. For further reduction of environmental lead exposure, this study was therefore conducted to study the possible household related factors for children's blood lead level in Taiwan.

In total, based on a stratified random sampling strategy, 934 kindergarten students were recruited from all around this island country from April to October 2011 in Taiwan after their parents have signed a statement of consent. Venous blood sample was drawn from each participant and analyzed for lead content with inductively coupled plasma mass spectrometry. Information on demographics and household environment of the study subjects were collected through questionnaire administration.

The geometric mean of lead level in the blood samples of study subjects was 1.86 μg/dL with geometric standard deviation of 1.55. Blood lead level was found negatively correlated with household income and parental educational levels ($p < 0.0001$). Study subjects whose fathers worked in the industries of agriculture, forestry and fishery tended to have relatively higher blood lead levels (> 2.20 μg/dL). Ethical activity like incense-burning at home was also identified a significant factor for blood lead level elevation ($p<0.0003$) along with a dose-response relationship with the frequency of incense-burning at home ($p=0.0051$).

Since health effects of low level lead exposure were continuously reported in recent years and currently no consensus was reached for a safety threshold for children blood lead level, any significant small, or even trivial, factor is deserved of being identified in order to further prevent children's lead exposure. Incense burning at home is one of the common traditional religion activities in Taiwan. Further effort is warranted to eliminate lead content in incense to further reduce lead exposure for the families with incense-burning at home.

12. Pregnancy & reproduction

2-75

THE IMPACT OF SEMINAL MINERAL ELEMENTS AND SEMINAL ZINC ON CONVENTIONAL SEMEN PARAMETERS OF TUNISIAN INFERTILE MEN

Atig Fatma1,2, Raffa Monia2, Kerkeni Abdel-Hamid2, Saad Ali1 and Ajina Mounir1

Unit of Reproductive Medicine, University Farhat Hached Hospital, 4000 Soussa, Tunisia
Research Laboratory of "Trace elements, free radicals and antioxidants", Biophysical Department, Faculty of Medicine, University of Monastir, 5000 Monastir, Tunisia

Introduction: Human seminal plasma contains several oligo-elements that play an important role in the normal function of sperm. Recent reports summarized the role of zinc (Zn), calcium (Ca) and magnesium (Mg) in human reproductive potential. It was reported that determination of Ma and Ca levels is usually associated to zinc levels. In fact, zinc has antioxidative properties and his absence can contribute to oxidative damages and consequently poor sperm quality. Intracellular Ca is essential for sperm motility and acrosomal reaction. Mg is involved in many biologic processes and has an important function in enzymatic reactions and ejaculation. The aim of this study consists to (1) determine levels of zinc, Mg and Ca in seminal plasma of subjects consulting for infertility problem and to (2) evaluate the effects of these elements on semen parameters (Motility, sperm count and typical forms).

Material and Methods: Semen samples were obtained from 206 patients presenting for infertility evaluation in the cytogenetic and reproduction biology department of Farhat Hached hospital of Soussa. After liquefaction, analysis of semen parameters was performed according to World Health Organization (WHO) criteria. Subjects were divided into four groups: normozoospermia (n=44), asthenozoospermia (n=79), teratozoospermia (n=59) and oligozoospermia (n=25). These specimens were designated for seminal zinc and mineral elements (Ca and Mg) analysis. Concentrations of these elements were determinated using flame atomic absorption spectroscopy (FAAS). The statistical analyses were done with the statistical package for social sciences (SPPS) for windows version 11.0.

Results: After semen analysis, concentrations of Zn, Mg, and Ca in the seminal plasma of all groups were determined by atomic absorption spectroscopy. For mineral elements, all infertile groups established significantly elevated seminal calcium levels than fertile men. Concerning, seminal concentrations of magnesium, we noted a significant decrease in all infertile subjects. Seminal Calcium showed a statistically significant correlation just to sperm motility ($P<0.001$) and sperm count ($P=0.002$). We noted a positive significant relationship between seminal magnesium and sperm count ($P=0.04$). Fertile subjects demonstrated higher seminal Zn levels than any infertile group and the difference was not significant. Seminal Zn correlated significantly with sperm motility ($P=0.05$) and normal morphology ($P<0.001$).

Conclusion: In conclusion, association of seminal Zn, Mg and Ca with basic semen parameters indicates that increase of seminal antioxidants is important to maintain a good quality of semen. Routine determination of Zn levels during infertility investigation is therefore recommended.

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IMPACT OF ZINC AND SELENIUM LEVELS IN FOLLICULAR FLUID ON THE OUTCOME OF ASSISTED REPRODUCTIVE PROCEDURES

Atig Fatma[1,2], Raffa Monia[2], Kerkeni Abdel-Hamid, Saad Ali[1] and Ajina Mounir[1]

Unit of Reproductive Medicine, University Farhat Hached Hospital, 4000 Soussa, Tunisia
Research Laboratory of "Trace elements, free radicals and antioxidants", Biophysical Department, Faculty of Medicine, University of Monastir, 5000 Monastir, Tunisia

Introduction: Follicular Fluid (FF) creates a relatively isolated compartment which provides oocytes with the specific microenvironment required for their maturation and function. Its organic composition includes non-enzymatic antioxidants such as "zinc and selenium" which serve as potential predictors of in vitro fertilization (IVF) outcome. The purpose of this study was to (1) determine the follicular fluid zinc, selenium and malondialdehyde acid (MDA) concentrations and to (2) evaluate their impact on the early embryonic development.

Material and Methods: Follicular fluid samples were recruited form 190 female partners (24-44 years) of couples undergoing IVF as an infertility treatment in our Unit of Reproductive Medicine (Soussa, Tunisia). Patients were divided into four groups based on the indications for IVF. Group I consisted of women with idiopathic infertility (controls [n=35]), group II: patients with tubal disease (n=50), group III: 50 women with endometriosis and group IV contained 55 patients from couples with male factor of infertility. After oocyte retrieval, specimens were centrifuged at 3500 rpm for 15minutes. The clear supernatants were divided in aliquots and frozen at -80°c until antioxidant and MDA analysis. For all the experiment, flame and furnace atomic absorption spectrophotometry were adopted for zinc and selenium determination; respectively. The amount of MDA was measured by thiobarbituric acid reaction method. Concerning IVF outcomes we determined the rates of early embryonic development. The statistical analyses were done with the statistical package for social sciences for windows version 11.0.

Results: In comparison between controls and patient groups (III and IV), zinc concentration showed significant increase (P<0.001) in controls (67,64 mg/l vs. 17,5 and 39,79 mg/l; respectively). Selenium concentrations were significantly higher (P<0.001) in control group compared to patients (patients II, III and IV). MDA which is the end product of lipid peroxidation, we noted elevated rates in the three patient groups (GII= 56,20 µmol/l, GIII=17,5 µmol/l and G IV=39,79 µmol/l) compared to control group (GI=0,006 µmol/l). Regarding the early embryonic development, all patient groups demonstrated a significantly decrease in comparison with controls. Correlations between non-enzymatic antioxidants and IVF outcome were positive and strongly significant. In fact, follicular zinc concentration was highly correlated to the oocyte maturity (r= 0.384**, P<0.01), the embryo fertilization (r=0.338**, P<0.001), the cleavage rate (r=0.347**, P<0.001) and embryo quality "grade I"

(r=0.269**, P<0.001). Meanwhile, there are significant and negative correlations noted between MDA levels and the fecundity parameters. On the other hand, we estimated correlations that can be exist between MDA and studied trace elements and we found high negative correlations with zinc(r=-0.340**, P<0.001) and selenium (r =-0.950, P<0.001).

Conclusion: To summarize, it has been shown that there are detectable levels of zinc and selenium in human follicular fluid and there is evidence of antioxidant activity of these trace elements. It was proved whether the non-enzymatic antioxidant status and the lipid peroxidation in the FF may play a role in the process of gamatogenesis and fertilization and they would be the best predictors of IVF outcome.

2-77

THE INSULIN GROWTH FACTOR SYSTEM AND PREGNANCY-ASSOCIATED PLASMA PROTEIN A IN RESPONSE TO LEAD EXPOSURE FROM CIGARETTE SMOKING

Magdalena Chelchowska1, Katarzyna Jablonka-Salach2, Joanna Gajewska1,Tomasz Mikolaj Maciejewski3, Jadwiga Ambroszkiewicz1, Teresa Laskowska-Klita1, Jan Barciszewski4

1 Screening Test Department and 3Department of Obstetrics and Gynecology, Institute of Mother and Child Kasprzaka 17A,, 01-211 Warsaw, Poland e-mail: lipidy.imid@imid.med.pl
2Faculty of Chemistry, University of Warsaw , Pasteura 1,02-093 Warsaw, Poland, e-mal: kjablonka@chem.uw.edu.pl
4Institute of Bioorganic Chemistry, Polish Academy of Science Poland, Noskowskiego 12/14, 61-704 Poznan, Poalnd, e-mail: Jan.Barciszewski@ibch.poznan.pl,

Objective. Limited studies have the examined the association between maternal low-level lead exposure, insulin growth factor system axis and fetal growth. Maternal smoking during pregnancy is associated with a reduction in birth size but the mechanism by which this occurs has not been adequately clarified. Therefore we examined the effect of lead exposure from cigarette smoking on insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II), binding protein BP-4 and pregnancy-associated plasma protein A (PAPP-A) in pregnant women. We also studied the correlations between these biochemical parameters and fetal growth.

Methods. Seventy healthy pregnant women, were divided into smoking and tobacco abstinent group according serum cotinine concentration. Smokers were defined as those women who reported their smoking habit as maintained at a rate of minimum 5 cigarettes per day during pregnancy

Results. The concentrations of lead in group of smoking women were statistically significant higher in serum (3.0µg/L vs 0.8µg/L; $p<0.01$) as well as in whole blood (19.7µg/L vs 14.1µg/L; $p<0.05$) than in group of tobacco abstinent. The same was observed in case of IGF-I, IGF–II ($p<0.05$) and PAPP-A concentration ($p<0.01$). There was a weak correlation between serum lead level and concentration of PAPP-A ($r=-0.39$; $p<0.05$) in the group of cigarette smokers. Birth weight in infants born of smoking mothers was lower in average by 300 g as compared with non-smoking ones ($p<0.05$) and correlated negatively with serum lead concentration ($r=-0.45$; $p<0.05$). In tobacco-abstinent women the concentration of IGF-II correlated positively with level of PAPP-A ($r=0,59$; $p<0.01$) and negatively with IGFBP-4 concentration ($r=-0,36$; $p<0.05$).

Conclusion. Exposure to low-level of lead from cigarette smoking affected insulin growth factor system and PAPP-A concentration in pregnant women, which can result in a significant reduction in weight of the newborn at birth.

2-78

THE BIOLOGICAL ROLE OF TRACE ELEMENTS IN THE PATHOGENESIS OF PREGNANT WOMEN WITH ALIMENTARY OBESITY

L A. Kovalchuk, A.E. Tarkhanova

Institute of Plant and Animal Ecology, Ural Branch Russian Academy of Sciences; Middle Urals Scientific Center Russian Academy of Medical Sciences, Laboratory of Adaptation Problems; The Ural State Medical Academy; Municipal hospital №1, Yekaterinburg;RUSSIA. kovalchuk@ipae.uran.ru

Presently the number of pregnant women with alimentary obesity in growing in all developed countries – from 16% to 27%. In Russia the number has grown up to 23% during the last 10 years and continues to grow. The participation of the essential trace elements during pregnancy has no doubt, therefore, their embryo- and fetotoxicity is an important problem. We investigated 96 pregnant women at the age of 23 to 34. Pregnancies had complications 75% (I stage of obesity) to 100% (III stage). The main complication were gestosis, danger of pregnancy interruption, frequent respiratory diseases; nephropathy of pregnancy was observed at all obesity stages. The analysis of trace elements in the blood serum revealed lower 25% Zn content (p=0.028), lower 23% Fe content (p=0.014), lower 53% Ca content (p=0.014), lower 33.3% Mg content (p=0.015). Concentration of Mn were 75% (p=0.0257). higher, those of xenobiotics: Cd (0.01 + 0.003 mkg/ml ; p=0.004) and Pb (0.16 + 0.02 mkg/ml ; p=0.0018) – reason for the disturbance of lipid metabolism. The disbalance of trace elements is pathogenetic link in the development of obesity. Futher investigation is necessary for the pathogenetic therapy of individual trace elements as specific agents of activation or disactivation of the main biochemical factors contributing to the pathological processes of obesity. Optimization of therapy by methods of metabolic correction is possible in practical medicine.

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2-79

ELEMENT PROFILES OF UMBILICAL CORD TISSUE AND PLACENTA AND THEIR USEFULNESS AS PREDICTORS OF PRENATAL EXPOSURE TO TRACE ELEMENTS IN THE GENERAL JAPANESE POPULATION

Mineshi Sakamoto [a,*], Akira Yasutake [a], Hing Man Chan [b], José L Domingo [c], Machi Kubota [d], Katsuyuki Murata [e]

The profiles of elements such as total mercury (T-Hg), inorganic mercury (I-Hg), lead (Pb), cadmium (Cd), selenium (Se), zinc (Zn), and copper (Cu) were assessed in human umbilical cord and placenta, in relation to their concentrations in maternal and cord red blood cells (RBCs). Maternal and cord blood RBCs, placenta and cord tissues samples were collected from 48 Japanese mother-child pairs immediately after birth. Cord and placenta tissues were rinsed with 0.9% saline, which was removed using paper towels. The tissue was then freeze-dried. T-Hg and I-Hg concentrations in tissues were determined by an oxygen combustion-gold amalgamation method using an atomic absorption mercury detector. Other metals were analyzed by ICP-MS. Pb, Cd, Zn, Cu, and I-Hg concentrations in placenta were significantly higher than those in umbilical cord. Particularly, Cd and I-Hg levels in the placenta were 66 and 2.5 times higher than those found in cord tissue, indicating that these metals were strongly trapped by the placenta. On the other hand, T-Hg concentration in cord tissue was 1.4 times higher than that in the placenta, indicating exceptionally higher placental transfer of methylmercury (MeHg) than that of other trace elements. The percentages of I-Hg in T-Hg were 4.5% in cord tissue and 14.5% in placenta, respectively. T-Hg in both placenta and cord tissue showed significant and strong correlations with those found in cord RBCs ($r = 0.93$, respectively), which are thought to be the best biomarker of fetal body burden of MeHg. Se concentrations in cord tissue and placenta showed significant, but moderate correlations, with that noted in cord RBCs ($r = 0.34$ and 0.54, respectively). However, Pb, Cd, Zn, and Cu levels in cord and placenta tissues showed no significant correlation with those in cord RBCs. These results suggest that T-Hg in placenta and cord tissue can be equally used as a good biomarker for prenatal MeHg exposure in newborns. Se in cord tissue and placenta can predict approximately 12% and 29% of fetal body burden of Se, respectively, while Pb, and Cd in these tissues will not be useful for predicting their prenatal body burdens.

2-80

SMOKING INFLUENCE ON THE ELEMENT STATUS OF WOMEN OF REPRODUCTIVE AGE

Kiyaeva E.V., Notova S.V., Skalny A.V., Alidzhanova I.E., Miroshnikov S.V., Lizurchik L.V.

Orenburg State University, Russia, 460018, Orenburg, prospect Pobedy, 13, E-mail: elena_sap@mail.ru

It is proved that reproductive function of the woman in many respects is defined by a favorable current of the puberty. Somatic diseases, food violations, addictions reflecting the element status during this period, can have the most negative consequences for reproductive health.

Girls at the age of 16-20 years, female students of Orenburg State University (90 people) were examined. Two groups (smokers, n=41, and non-smokers, n=49) were sorted out. Hair samples were used to study the trace element status of the body. The elemental composition of hair identification was performed at the Center for Biotic Medicine (Moscow).

Comparison of the element status of smokers and not smokers revealed an imbalance in essential chemicals in the group of smoking girls. The decrease in the content of iodine ($p \leq 0,05$) by 2,2 times, selenium ($p \leq 0,05$) by 1,7 times and zinc by 1,3 times was shown. These elements participate in regulation of reproductive function. The increase of the content of calcium, potassium and magnesium in hair of the surveyed smokers and the decrease in the content of vanadium and phosphorus were also observed.

The work was supported by the Ministry of Education and Science of the Russian Federation (Project № 1.5.11).

2-81

ASSOCIATION OF ELEVATED SERUM FERRITIN LEVEL AND THE RISK OF GESTATIONAL DIABETES MELLITUS IN LIBANESE NONANEMIC PREGNANT WOMEN

Salam Zein[1,2] Samar Rachidi Sanaa Awada , Amal Al-Hajje[1] , Pascale Salameh[1] Isabelle Hininger-Favier[2,3]

[1] *Laboratoire de Recherche Clinique et Epidémiologique – Faculté de Pharmacie – Université Libanaise – Beyrouth – Liban*
[2] *Université Joseph Fourier, Laboratoire de Bioénergétique Fondamentale et Appliquée, Grenoble, France*
[3] *Inserm U1055, Grenoble, France*
salamzein@hotmail.com

During pregnancy, the insulin resistance is a physiological phenomenon that when it is not managed can become pathological and called gestational diabetes mellitus (GDM). Emerging data from an array of epidemiological studies support the significant association between serum ferritin, the most frequently studied measure of body iron stores, and GDM. Multiple mechanisms that link iron with abnormal glucose metabolism have been proposed, possibly mediated through oxidative stress. The objective of the present work was to study in nonanemic women the relationship between a high initial antenatal serum ferritin level and the occurrence of GDM in the third trimester of pregnancy. This prospective study involved 110 Lebanese nonanemic (Hb ≥ 11g/dL) healthy pregnant women recruited at the first visit of pregnancy. The participants were divided into 2 groups based on maternal ferritin levels at the booking visit. Data on socioeconomic status, demographics, lifestyle and food habits were obtained by interview. The prevalence of GDM, diagnosed according to the American Diabetes Association criteria, and the oxidative stress markers were evaluated at 24 to 28 weeks of gestation and at delivery. The anthropometric and apgar score of the babies will be related to ferritin level and oxidative stress parameters of the mothers.

For statistical analysis, the Student *t* test and the Chi-square test were used to compare means for continuous variables or frequencies for discrete variables, respectively. Multivariable logistic regression models were used to derive estimates of Odds ratios and 95% CIs. According to our hypothesis, we expect to observe a correlation between a high ferritin status, oxidative stress parameters and the risk of GDM. These results could have a negative impact on Apgar score. If ours hypothesis were confirmed, these results could be of ~~a~~ great interest for recommendation regarding the initial ferritin level in pregnancy to prevent the risk gestational diabetes.

13. *Dermatology*

2-82

PROSPECTIVE STUDY ON THE ROLE OF TRACE ELEMENTS IN PSORIASIS

J Tarekelmimr

***Background**:* Psoriasis is a chronic, recurrent skin disorder characterized histologically by cutaneous inflammation, increased epidermal proliferation, hyperkeratosis, angiogenesis, abnormal keratinization, shortened maturation time and parakeratosis. Data on the involvement of trace elements in the pathogenesis of psoriasis is limited.

***Objectives**:* To evaluate and provide an insight about the possible role of the trace elements in the etiopathogenesis and the extent of psoriasis.

***Methods**:* Serum levels of 21 trace elements namely Lithium, Boron, Rubidium, Manganese, Iron, Zinc, Copper, Selenium, Cadmium, Magnesium, Aluminum, Potassium, Chromium, Cobalt, Bromine, Tin, Mercury, Sodium, Calcium, Cesium, Iodine were analyzed in the serum samples of 30 patients with psoriasis patients and 10 healthy volunteers served as a control group. Patients were clinically evaluated by Psoriasis Area Severity Index (PASI) score of skin lesions presented at the time of blood collection. Trace element analysis was carried out by using an inductively coupled plasma optical emission spectrometry (ICP/ OES).

Results: There were a highly significant decrease in serum level of lithium, boron, cadmium and iron, and there were a highly significant increase in serum level of rubidium, manganese, zinc, copper and selenium in patients in comparison to control group. And there was non significant difference in the remaining elements (magnesium, aluminum, potassium, chromium, cobalt, bromine, tin, mercury sodium, calcium, cesium, iodine) in patients compared to controls.

***Conclusions**:* There is a disturbance in the element contents and also element–element interdependency in psoriasis patient's serum when compared to controls. These elements may serve as biomarkers for the disease as a prognostic tool and normalization of these elements may serves as a parameter of the efficacy of the treatment.

2-83

STUDY OF THE ROLE OF SOME TRACE ELEMENTS IN ACNE VULGARIS

T. Elnimr, tarekelnimr@gmail.com

Background: Acne is a common disorder affecting the pilosebaceous unit, clinically characterized by the presence of comedones, inflammatory papules, pustules and sometimes, nodules and cysts arising commonly during adolescence and causing great psychosocial stress. Diet may be an important factor, particularly in mediating the inflammation and oxidative stress of the acne process.

Objectives: To evaluate and provide an insight about the possible role of some trace elements in the etiopathogenesis and treatment of acne vulgaris.

Patients & Methods: Serum and urine levels of some trace elements namely zinc, selenium, chromium, manganese, lithium and copper were analyzed in the serum and urine samples of 30 patients with acne vulgaris and 10 healthy volunteers served as a control group. Patients were clinically classified into 4 groups, using a simple grading system. Each group was subjected to treatment with zinc tablets for 4 weeks to assess the effect of the drug. Trace element analysis was carried out by using an inductively coupled plasma optical emission spectrometry (ICP/ OES).

Results: There was a highly significant decrease in serum and urine level of zinc, lithium and in serum level of chromium. There was a highly significant increase in serum and urine level of manganese and copper in acne patients in comparison to control group. While, there was non significant difference in serum and urine level of selenium and urine level of chromium in patients compared to controls.

Conclusion: There is a disturbance in the element contents and also element–element interdependency in acne patients. These elements may serve as biomarkers for the disease as a prognostic tool and normalization of these elements may serve as a parameter of the efficacy of the treatment.

2-84

VALIDATION OF ANALYTICAL PROCEDURE FOR THE DETERMINATION OF ALUMINIUM USED IN ANTIPERSPIRANTS ON HUMAN SKIN IN FRANZ^{TM} DIFFUSION CELL

1Guillard O., 2Fauconneau B., 1Marrauld A., 3Deloncle R., 4Pineau A.,

1CHU Poitiers, Department of Biochemistry, 86021, Poitiers, France,
2CHU Poitiers, Department of Pharmacology, 86021 Poitiers, France,
3Tours University, Department of Toxicology, 3700 Tours, France,
4Nantes University, Department of Toxicology, 44000 Nantes, France,

A local case report of hyperaluminia (aluminium concentration : 3,88 mol/L – N : < 0,3 mol/L) in a woman using an aluminium-containing antiperspirant for 4 years raises the question of possible transdermal uptake of aluminium salt as a future public health problem. Given a lack of in vitro data, it became necessary to validate an analytical procedure for the determination of aluminium (Al) in commercialized cosmetic formulas used in antitranspirants on human skin in FranzTM diffusion cell. (1,76 cm2). Percutaneous absorption was studied quantitatively on human full skin biopsies bathing in a saline solution (Receptor fluid). All measurements were performed in a clean room by Zeeman Electrothermal Atomic Absorption Spectrophotometry. The analytical validation was performed on Receptor fluids and skin digested solutions. The detection and quantification limits were set at 3g/L.

Precision analysis for Al as within-run (n=12) in Receptor fluids were 2.73 ,6.36 and 3.96 (CV %) for 16, 120 and 220 g/L respectively. The between- run (n=68) for the same solutions were 3.90 , 4.00 and 5.20 (CV %) respectively. For mineral tissue residues on three different concentrations (15, 30 and 120 g/L), within- run (n=12) were 4.00 ,1.60 , and 4.20 respectively and between- run (n=15) 5.80 , 4.10 and 4.70(CV %).

This analytical validation under standardized in vitro conditions allowed to perform the measurement of human percutaneous absorption and skin distribution of aluminium chlorohydrate in common cosmetic formulations.

2-85

TRANSDERMAL AL UPTAKE FROM ANTIPERSPIRANTS THROUGH HUMAN SKIN IN THE FRANZ™ DIFFUSION CELL

[1]Pineau A., [2]Guillard O., [2]Marrauld A., [3]Deloncle R., [4]Fauconneau B.

[1]Nantes University, Department of Toxicology, 44000 Nantes, France
[2]CHU Poitiers, Department of Biochemistry, 86021, Poitiers, France
[3]Tours University, Department of Toxicology, 3700 Tours, France
[4]CHU Poitiers, Department of Pharmacology, 86021 Poitiers, France

Subsequent to the validation of the analytical procedure for the aluminium (Al) assay with different media on human skin using a Franz™ diffusion cell, an *in vitro* study was initiated by the French Health Product Sanitory Safety Agency (ANSM ex AFSSAPS) on human skin as concerns possible cutaneous penetration on healthy and stripped skin of different commercials Al-containing antiperspirants formulas (aerosol-base, roll-on and stick). This study was carried out in accordance with applicable OECD procedures (428 guideline 2004).

The Al assays were performed in a clean room by Zeeman Electrothermal Atomic Absorption Spectrophotometry. Following contacts lasting 6, 12 and 24 hours on human viable skin (n=5 donors), the Al assays showed only insignificant transdermal absorption of Al (≤ 0,07 % of the quantity of Al deposited) and particulary low cutaneous quantities that varied according to the formulations (1.8 µg/cm² for aerosol base and stick – 0,5 µg/cm² for the roll-on). On stripped skin, for which only the stick formulation was tested, the measured uptake was significantly higher : 11.50 µg/cm² versus 1.81 µg/cm² for normal skin. These new findings convincingly show that exposure to antiperspirants commercial products presenting with usual 2,5 % of tolerable Al fails to ensure consumer safety, especially in cases of frequently repeated used on damaged or injured skin.

So, ANSM strongly recommends restriction in the concentrations of Al cosmetics products to 0,6 % (as expressed in Al).

2-86

SUBCUTANEOUS PSEUDOLYMPHOMA : ALUMINIUM OVERLOAD AFTER 5 YEARS IN SKIN BIOPSY FOLLOWING POST-VACCINATION.

[1]Guillard O., [2]Fauconneau B., [3]Pineau A., [4]Deloncle R., [5]Bellocq JP ., [5]Chenard MP

[1] *CHU Poitiers, Department of Biochemistry, 86021, Poitiers, France,*
[2] *CHU Poitiers, Department of Pharmacology, 86021 Poitiers, France,*
[3] *Nantes University, Department of Toxicology, 44000 Nantes, France,*
[4] *Tours University, Department of Toxicology, 3700 Tours, France,*
[5] *CHU Strasbourg, Department of Pathology, Haut Pierre Hospital, 67098 Strasbourg, France*

Aluminium (Al) hydroxide is currently used as an effective adjuvant in a wide range of vaccines. Al compounds are well-known for enhancing immune response to the antigen and allergen. In 2005, it was reported on a first demonstration of vaccination-derived Al deposit in lesions of cutaneous lymphoid hyperplasia. Our patient, many years after vaccination, presented a subcutaneous pseudolymphoma in site of vaccine injection. In skin biopsy Al deposits are assessed by Morin stain and quantification of Al is performed in a clean room by Zeeman Electrothermal Atomic Absorption Spectrophotometry. Morin stain shows Al deposits in the macrophages and Al assays (in µg/g, dry weight) were 768.10 ± 18 for the patient versus 5.61± 0.59 and 9.13 ± 0,057 for two controls. Given the pathology of this patient and the high Al concentration in skin biopsy, the authors wish to draw attention when using the Al salts known to be particulary effective as adjuvants in single or repeated vaccinations.

2-87

ANTAGONIST EFFECTS OF SELENIUM SUPPLEMENTATION AS A FUNCTION OF CHEMICAL FORM AND DOSE: ANTIOXIDANT VERSUS TOXIC EFFECTS.

F Hazane-Puch*, P Champelovier[£], J Arnaud*, C Garrel*, F Laporte* and P Faure

** Unité de Biochimie Hormonale et Nutritionnelle, Département de Biochimie, Toxicologie et Pharmacologie*
[£] Unité de Cytologie, Département d'Anatomie et de Cytologie Pathologiques
Institut de Biologie et de Pathologie, Centre Hospitalier Universitaire de Grenoble, CS 10217, F-38043 Grenoble

Background: The biological effect of selenium (Se) is modulated by different factors such as the baseline patient Se status, the dose, chemical form and duration of the supplementation. Therefore, the anticancer effects of Se, particularly skin cancer remain unclear.

Objectives: The aim of this work was to investigate Se antagonist effects as a function of chemical form and dose in normal human skin fibroblasts (NHSFs). The Se antioxidant protective effects versus toxic effects triggering cell death were explored.

Methods: Two selenium species were used: an inorganic (sodium selenite, Na_2SeO_3) and an organic (selenomethionine, SeMet) forms. Cells were incubated with RPMI-1640 supplemented with 10% FCS and Na_2SeO_3 or SeMet for six days in concentration from 0.1 to micromolar range. Se absorption was determined. At non toxic doses, glutathione peroxidase 1 (GPX1) activity, selenoprotein W1 (SEPW1) and matrix metalloproteinases (MMP) -1 and -3 transcript expressions, thiol-groups and malondialdehyde (MDA) concentrations were measured. At toxic doses, cell cycle and cell death were characterized.

Results: Primary results indicated that SeMet was better uptaked than Na_2SeO_3. At non toxic doses, both forms induced GPX1 and SEPW1, the induction depending on the dose. Na_2SeO_3 induced MMP1 whereas SeMet decreased MMP3. On the contrary, the two forms did not modify MDA or thiol-groups concentrations Na_2SeO_3 toxicity was much higher than SeMet, with IC50 at 6.6 and 62.0 µM respectively. At toxic doses, Na_2SeO_3 inhibited cell proliferation associated with a blockage in the S-G2 phase of the cell cycle and induced DNA fragmentation leading to apoptosis cell death via caspase-3 activation.

Conclusion: Differences in terms of protection and antioxidant effects as well as toxicity were evidenced according to selenium forms and doses. Despite the translation from cellular model to human study needs further steps; our results confirmed that the dose must be adapted to the chemical form.

2-88

EVALUATION OF SELECTED HEAVY METALS IN COSMETICS USING ATOMIC ABSORPTION SPECTROMETRY

Adnan M. Massadeh[a,*], Mohammad Y. El-khateeb[b], Shaza M. Ibrahim[b]

[a] *Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, P. O. Box 3030, Irbid 22110 Jordan.*

[a,b] *Department of Chemistry, Faculty of Science and Arts, Jordan University of Science and Technology, P. O. Box 3030, Irbid 22110 Jordan*

** Corresponding author: E-mail address: massadeh@just.edu.jo, Fax: 00962-2-7201063*

Heavy metals (Cd, Cr, Cu, Ni and Pb) contents of cosmetic samples from Jordan, Sudan and Syria markets are determined by Atomic Absorption Spectrometry (AAS). The results revealed that the range concentrations of heavy metals in cosmetic samples from Jordan market were: Cd (0.03-0.10) µg/g, Cr (0.0-1.00) µg/g, Cu (0.60-7.4) µg/g, Ni (0.5- 3.6) µg/g and Pb (0.3-15.4) µg/g. Whereas, in Sudan market, the heavy metals concentrations were Cd (0.01-0.15) µg/g, Cr (0.0-27.9) µg/g, Cu (0.60-10.1) µg/g, Ni (0.00- 5.7) µg/g and Pb (0.02-3.8) µg/g. Also, the range concentrations of heavy metals in Syria market were: Cd (0.04-0.056) µg/g, Cr (0.243-0.834) µg/g, Cu (0.611-1.27) µg/g, Ni (0.734- 1.41) µg/g and Pb (4.85-27.7) µg/g. Moreover, results revealed that kohl samples have high concentration of most of the studied metals, particularly Pb. ANOVA analysis indicated that there is a significant difference in heavy metal levels for samples obtained from Jordan and Sudan markets.

14. Toxicology

2-89

DISTURBED HOMEOSTASIS OF SOME INORGANIC ELEMENTS ASSOCIATED WITH CHRONIC EXPOSURE TO LOW LEVELS OF BENZENE AND POSSIBLE ASSOCIATED HEALTH HAZARDS

El-Bassiouni EA, *Ramadan HS, * Kotb MA.

Dept. of Pharmacology
**Dept. of Bio-Medical physics*
Medical Research Institute, Alexandria University, Egypt
El-Bassiouni,(elbassiouni@hotmail.com- Mobile : 01001589725)
Ramadan HS (elfarouk22005@yahoo.com. Mobile : 01120718722)
Kotb MA (metkotb45@gmail.com. - Mobile : 01061034502)

Moderate and long-term exposures to benzene carry the risk of numerous health problems. Involvement of inorganic elements in the mechanisms of initiating such hazards has been suggested, but their role in problems resulting from occupational exposure to benzene has not been reported. Imbalance in homeostasis of such elements may help explain the disturbance in some biological functions observed in exposed personnel.

The present study was undertaken to investigate these possibilities in an all male cohort of 40 healthy control subjects and 110 individuals occupationally chronically exposed to low-levels of benzene in their daily activity. Benzene-exposed workers were found to be under oxidative stress with significant elevation of plasma MDA level, decrease in plasma total antioxidant activity and a tendency for a higher degree of hemolysis in their blood samples. Nineteen elements were determined in plasma and RBCs of these samples: lead, mercury, copper, cobalt, chromium, selenium, cadmium, aluminium, rubidium, magnesium, manganese, zinc and tin, as well as sodium, potassium, lithium, calcium, phosphorus and boron.

Higher levels of lead, probably from leaded gasoline, as well as mercury and cadmium were found in the plasma and RBCs of benzene-exposed workers, while the levels of zinc, selenium and copper were lower. Cobalt showed only a small but statistically significant increase. There were no significant differences between other assayed elements in exposed workers and control subjects.

All of the accumulated metals are proposed to contribute to oxidative stress by different mechanisms. They are either redox-active and directly involved in the production of free radicals, or are redox-inert, and contribute to oxidative stress by inhibiting antioxidant defense. The effect of excessive production of free radicals on RBCs membrane may explain the observed tendency for hemolysis in blood samples from benzene-exposed subjects. Metals with decreased concentrations like zinc and selenium may contribute to other metabolic disturbances and accelerate free radical production, probably through decreased participation in antioxidant protection.

2-90

ASSESSEMENT OF NATURAL RADIOACTIVITY AND CHEMICAL COMPOSITION LEVELS FROM PHOSPHATE LAYERS IN TUNISIA

K. Mounira[1*], F. Gharbi[2] , H. Baltas [3,] H. Hamed[4], B.S. Ridha[5]

*1*Faculty of Medecine of Sousse, Unit of biophysics - k.mounira@gmail.com*
2 National Centre of Nuclear Sciences and Technology, Sidi Thabet, Tunisia foued.gharbi@cnstn.rnrt.tn
3 Recep Tayyip Erdogan University, Departement of physics, Rize, Turkey - hbaltas@ktu.edu.tr
4 Company of Phosphates of Gafsa, Research Center of Métlaoui, Tunisia - hbib.hamed@cpg.com.tn

Faculty of Medecine of Sousse, Unit of biophysics
istmtrbs@yahoo.fr

The chemical composition and the natural radioactivity levels of 226Ra, 232Th and 40K, in phosphate layers samples collected from Gafsa deposit have been determined using the X-ray flourescence (XRF) and gamma spectrmetery, respectively. The naturally occurring 226Ra, 232Th and 40K from Moulayres ranged from 360.918-580.204 Bq/kg, 12.509-70.638 Bq/kg, 21,625- 149,437 Bq/kg and 20,282- 85,757 Bq/kg respectively. The 226Ra, 232Th, 40K and 235U activities from Kef Echfayer are 285.664-447.95 Bq/kg, 26.898-76.436 Bq/kg, 15.188-28.766 Bq/kg and 6.902-24.85 Bq/kg, respectively. In addition, absorbed dose rate in air (D), annual effective dose (AED), radium equivalent activities (Raeq) and external hazard index (Hex) associated with the natural radionuclide are calculated to assess the radiation hazard of the natural radioactivity in the phosphate layers samples. The concentrations of the natural radionuclides and the chemical in studied samples were compared with the corresponding results of different countries.

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THERAPEUTIC EFFECT OF CARROT PECTIN ON LEAD ACETATE EXPOSED RENAL AND HEPATIC SYSTEM : BIOCHEMICAL AND HISTOPATHOLOGICAL STUDY

Ouardia Ouldali1,2, A. Aoues2, B. Meddah1, M. Slimani2,

1 Department of Biology, Laboratory for Research on Biological Systems and Geomatics, Faculty of Nature and Life Sciences, University of Mascara, Algeria
2 Department of Biology, Laboratory of Experimental Biotoxicology, Biodepollution and Phytoremediation, Es-Senia University, Oran, Algeria
oouldali@yahoo.fr

The poisoning by ingestion of lead containing products is still an important toxicological concept in pediatric age group. In subacute lead poisoning, decontamination options are limited since administration of chemical chelators (EDTA, DMSA, and others) in efficacy administration of chemical chelators (EDTA, DMSA, and others) as curative treatments. This two-step study is designed to study the effect of pectin, which is a natural inert adsorbent, the chelation of lead and reduce the toxic effect in rats.

The adverse effects of lead toxicity in the liver and kidney concerned, more precisely, the increased activity of transminases and the renal markers: Urea, Creatinine have been demonstrated after 1 month of oral lead administration. The introduction of carrot pectin to a level of 3% in the feeding of intoxicated rats has shown a chelating and correcting effect on liver and kidney disturbances caused by lead toxicity, which is reflected by a significant decrease ($P<0.05$) of AST (from 107 to 90.89 to 80,03 UI/l), ALT (from 48,06 to 35,37 to 37,09 UI/l), PAL (from 210,97 to 195,63 to 191,94 UI/l), Urea (from 0,60 to 0,53 to 0.50 mmol/l) and creatinine (from 15.51 to 13.42 to 10.15 mmol/l) of the treated rats by pectin as compared with the untreated groups. The findings of present study suggest the possible oxidative stress and histopathological abnormalities induced by lead can be neutralized by pectin in the lead exposed population.

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CONJUGATED DIENES OF LINOLEIC ACID (CLA) CHANGE THE MINERAL COMPOSITION OF MICE BONES

Gutowska Izabela[1], Baranowska-Bosiacka Irena[2], Szynkowska Agnieszka[1], Jakubczyk Karolina[1], Siwiec Ewa[1], Noceń Iwona[3], Rybicka Marta[1], Chlubek Dariusz [2], Stachowska Ewa[1]

[1]Department of Biochemistry and Human Nutrition, [2]Department of Biochemistry, [3]Department of Medical Chemistry, Pomeranian Medical University, Szczecin, Poland
e-mail: gutowska@pum.edu.pl

Since the role of conjugated dienes of linoleic acid (CLA) in the weight loss process was documented, they have become the main ingredient in slimming dietary supplements. CLAs containing diet supplements are usually a mixture of two linoleic acid isomers: trans-10 cis-12 CLA and cis-9, trans-11 CLA, contained in equal proportion 1:1. Each of the isomers can exert an opposite biological action in many aspects. But only the trans-10, cis-12 CLA acts as the factor implicated in the adipocites delipidation process in vivo, leading to the fat tissue reduction. The second isomer cis-9, trans-11 does not exert delipidation effect both in vitro and in vivo.

Aim of work: The actual influence of CLAs on body mass reduction has not been clearly explained. There are still missing data in medical studies concerning the CLAs influence on the micro- and macroelements metabolism in various tissues. That fore we decided to examine influence of conjugated dienes of linoleic acid (CLA) on fluoride accumulation in bones of mice.

Material and method: In the research we used bones and serum of 6 weeks old mice, which were feeding using MURIGRAN fodder including 6.5% of soy olive (control group) or 5.5% of soy olive with 1% CLA supplementation (examined groups: *cis*-9, *trans*-11 CLA; *trans*-10, *cis*-12 CLA; CLA mix 1:1; LA) during four weeks. Fluoride concentration was determined with the ionselective electrode; calcium and magnesium concentrations with the atomic absorption spectrometry. The results of the study were analyzed with Mann-Whitney U test. The significance level was $p \leq 0{,}05$.

Result: Mice supplementation with various isomers of linoleic acid (*cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA, CLA mix 1:1, LA) caused changes in bone mineral composition and the greatest changes were observed in the case of isomer *cis*-9, *trans*-11 CLA, which does not exert delipidation effect both *in vitro* and *in vivo* but is added to the slimming diet supplements in 1:1 proportion.

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FIXATION OF LEAD BY LACTIC ACID BACTERIA IN LIQUID MEDIUM

Akhmetsadykova Sh. 1,2,3, Loiseau G1., Konuspayeva G.4, Baubekova A.2,3, Akhmetsadykov5 N., Faye B. 2

1 *UMR Qualisud, CIRAD, TA B-95/16, 73, rue J.-F. Breton, 34398 Montpellier Cedex 5, France, a_shinara@yahoo.com*
2 *CIRAD - Département Environnements et Sociétés, Campus International de Baillarguet, TA C-DIR / B 34398 Montpellier, Cedex, France, faye@cirad.fr*
3 *Scientific-Production Enterprise "Antigen", 4 Azerbayev street, 040509 Almaty region, Kazakhstan, almagul_b@mail.ru*
4 *Al Farabi Kazakh National University, 71 av. Al Farabi, 050040 Almaty, Kazakhstan, gaukhar.konuspayeva@kaznu.kz*
5 *Kazakh National Agrarian University, 8 av. Abai 050013 Almaty, Kazakhstan, nurlan.akhmetsadykov@gmail.com*

Central Asia is known for its environmental pollution, and contaminants concentrate throughout the food chain. Thus, heavy metals as lead could be found in animal products as camel milk which is consumed in high quantity, especially in Kazakhstan. To test the detoxification effect of fermented camel milk, two stages study was performed: (i) isolation and identification of lactic bacteria (LAB) strains from fermented camel milk (shubat); (ii) quantitative lead fixation test by the isolated LAB. Identification was achieved by molecular methods (rRNA16S). The test consisted to put in presence a known quantity bacterial biomass of each of 13 strains tested with a known quantity of lead in liquid medium. Lead was added as $Pb(NO_3)_2$ at concentrations: 0, 1, 15, 30 and 50 mg/L. The concentration of unfixed lead was measured by atomic absorption spectrometry. According to results of sequencing, the majority of strains were of genus Enterococcus and Lactobacillus, secondarily Lactococcus and Leuconostoc.

All tested strains fix lead, but with different capabilities. For some strains saturation phenomenon was observed: the percentage of fixed Pb decreased when the concentration of lead increased.

Strains showing very high binding capacities were Enterococcus faecium, E. durans, L. kefiri and Leuconostoc mesenteroides..

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TO THE CONCEPT FORMATION OF THE CELLULAR TRANSPORT ROLE IN HEAVY METAL TOXICITY

Leonid M. Shafran*, Elena G. Pychteeva**

Ukrainian Scientific and Research Institute of Medicine on Transport
Kanatnaya Str., 92. 65039, Odessa. Ukraine - shafranlmo9@rambler.ru
Tel.: (+38048) – 728-39-73; Fax: (+38048) – 726-47-93
**Dr.med. sci., Prof., First Vice-Director of the Institute,*
Director of Hygiene and Toxicology Branch
***Cand. Chem. Sci., Manager of the Laboratory of Industrial and Environmental Toxicology*

A concept of toxic heavy metals (THM) cellular transport is proposed. It covers a two-fold contradictory system of metal detoxification or metallopaty formation. 1. A highly complex system of cellular transport THM includes membrane and kompartment located transport and auxiliary proteins, essential metals (EM) and metalloproteins, providing a consistent flow of all phases of indwelling of metal suction to excretion. 2. Transport and auxiliary proteins perform binding, reduced bioavailability, localization and elimination of THM. 3. EM transport systems are used to transfer the THM, as they don't have own. The normal homeostasis of EM prevents and disgomeostaz promotes entry into the cell and the THM toxic effect implementation. 4. THM entry into the cell contribute to their ionic and molecular mimicry, the closeness of the ionic radii and electrochemical potentials with EM. 5. Metalloproteins perform the transportation and the regulatory functions of the transmembrane revenues, compartmentalization and excretion THM from the cell. 6. The transport mechanisms involved in acute or chronic action depend of the concentration of incoming THM and performs different effects. 7. The biological effect of each THM has its own threshold: up to a certain intracellular concentration its toxicity not manifested due to sufficient metall binding capacity of competent proteins and high EM/THM ratio. 8. Intercompartment cell differentiation of THM concentrations is dynamic and depends on intensity of the inductive synthesis of transport proteins and cell functional state. 9. The lysosomes involving in the THM biotransformation and excretion is connected with the concentrations of metalloproteins' complexes in the cytoplasm and in proportion to the activity of lysosomal proteases. The excess of the complex above a certain threshold manifests inhibitory toxic effects. 10. Specificity of the toxic effect of THM determined by biotropic properties of transport proteins, their organospecificity, targeted synthesis, biodynamic and decomposition.

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ENVIRONMENTAL CONTAMINANT BISPHENOL A TOXICITY STUDY IN SWISS ALBINO MICE MODEL

Sameya Anjum and Sheikh Raisuddin

Jamia Hamdard, New Delhi, India

Bisphenol A (BPA) enters environment as an industrial or domestic waste or byproduct of industrial processes. BPA is a monomer of polycarbonate plastic used to manufacture plastic baby bottles and lining of food cans. It has endocrine-disrupting potential and exerts both toxic and estrogenic effects on mammalian cells. The aim of this study was to investigate BPA induced oxidative stress and toxicity in the testicular mitochondria of adult male mice. Mice were exposed to standardized dose of BPA (5, 10, 100 mg/kg body weight), orally for 14 days. BPA caused lipid peroxidation (LPO) and decrease in reduced glutathione (GSH) content of testicular mitochondria. Significant differences $P < 0.01$ was observed in the LPO and GSH parameters when compared with control values. BPA caused significant decrease in activities of marker mitochondrial enzymes such as succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase as compared to control group. Besides, it also affected activities of antioxidant enzymes such as superoxide dismutase, glutathione reductase and glutathione peroxidase. Significant differences $P < 0.01$ was observed in the SOD, GR and GPx parameters when compared with control values. These effects were increased as the dose of BPA was increased. Ultra structural changes observed by transmission electron microscopy showed BPA caused abnormalities like deformed acrosome and nucleus of spermatids and apoptotic cells were observed in the testes of treated animals. Hence we can conclude that BPA has induced oxidative stress in testicular mitochondria of exposed group and the results were further confirmed by the observations of transmission electron microscopy.

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EFFECTS OF ZINC OXIDE NANOPARTICLES (ZN O) AND/OR ZN CL² ON BIOCHEMICAL PARAMETERS AND TRACE ELEMENT LEVELS IN RAT LIVER AND KIDNEY

Salem Amara[1], Imen Ben Slama[1], Imen Mrad[1], Karim Omri[2], Jaber el Ghoul[2], Lassaad EL Mir[2,3], Khemais BEN Rhouma[1], Hafedh Abdelmelek[1] and Mohsen Sakly[1].

[1]Laboratoire de Physiologie Intégrée Faculté des Sciences de Bizerte, 7021 Jarzouna, Tunisia
[2]Labo. of Physics of Materials and Nanomaterials applied at environment, Gabes College of Sciences
[3]Al Imam Mohammad Ibn Saud Islamic University (IMSIU), College of Sciences, Department of Physics, Riyadh 11623, Saudi Arabia. Phone: 0021652322418, Fax : 0021672590566
Corresponding Author: Dr Salem AMARA
Laboratoire de Physiologie Intégrée, Faculté des Sciences de Bizerte, Jarzouna 7021, Tunisia
amara_salem_fsb@yahoo.fr

Background: The increased presence of nanoparticles in the environment suggests that a fundamental understanding of their mode and range of toxicity is needed.

Aim of the study : The aim of this study was to assess the potential sub-acute toxicity of ZnO nanoparticles (ZnO-NPs) in wistar rats by comparison to reference toxicant, zinc chloride ($ZnCl_2$) in a non-nanoparticulate form. We therefore studied the relationships between tissues zinc accumulation, the mineral status and plasmatic biochemical parameters.

Methods : Rats received ZnO-NPs and/or $ZnCl_2$ solution (25 mg/kg body weight) by intraperitoneal injection every other day during ten days.

Results : Sub-acute administration of ZnO-NPs and $ZnCl_2$ solution increased significantly zinc accumulation in rat liver. Howerver, zinc concentration in the kidney remained unchanged. Morover, the contents of trace elements in the liver and kidney were slightly modulated after ZnO-NPs and/or $ZnCl_2$ exposure. The combined injection of ZnO-NPs and $ZnCl_2$ increased the AST activity and uric acid concentration. By contrast, the same treatment decreased blood glucose levels. Interestingly, the analysis of the lyophilized powder of liver using the X-ray diffractometer showed the degradation of ZnO-NPs in ZnO-treated group, instead there is a lack of nanoparticles zinc oxide biosynthesis in $ZnCl_2$ injected rats.

Conclusions : This investigation suggests that combined injection of ZnO-NPs and $ZnCl_2$ has a possible toxic effect which could be related to Zn^{2+} release and accumulation in organs. Our findings provide crucial information that ZnO appears to be absorbed in the organs in an ionic rather than in a particulate form.

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CR(III) EXERTS STRONGER STRUCTURAL EFFECTS THAN CR(VI) ON THE HUMAN ERYTHROCYTE MEMBRANE AND MOLECULAR MODELS

Mario Suwalsky, Faculty of Chemical Sciences, University of Concepciòn, Concepciòn, CHILE - *msuwalsk@udec.cl*, Ricardo Castro, Faculty of Chemical Sciences, University of Concepciòn, Concepciòn, CHILE - *ricastro@udec.cl*, Fernando Villena, Faculty of Biological Sciences, University of Concepciòn, Concepciòn, CHILE - *fvillena@udec.cl*

Chromium exists in many oxidation states, of which only the hexavalent Cr(VI) and the trivalent Cr(lll) are stable under environ mental conditions. It is generally reported that Cr(VI) is highly toxic while Cr(lll) is relatively innocuous. With the aim to better understand the molecular mechanisms of the interaction of Cr (III) and Cr(VI) with cell membranes, $CrCl_3$ and K_2CrO_4 were lncubated with intact erythrocytes, isolated unsealed human erythrocyte membranes (lUM), and molecular models of the erythrocyte membrane. These consistee in bilayers built-up of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), phospholipid classes present in the outer and inner monolayers of the erythrocyte membrane, respectively. The capacity of Cr(lll) and Cr(VI) to perturb the bilayer structures of DMPC and DMPE were evaluated by X-ray diffraction. DMPC large unilamellar vesicles (LUV) and IUM were studied by fluorescence spectroscopy, and intact human erythrocytes were observed with scanning electron microscopy. In ail these systems, it was found that Cr(III) induced considerably higher structural effects than (Cr(Vl). These results indicate that interaction of Cr^{3+} ions with phospholipid bilayers perturb the bilayer structure. Since the lipid bilayer ls the membrane main permeability barrier, the structural perturbation will affect its permeability. It might also affect the functions of ion channels, receptors and enzymes immersed in the membrane lipid bllayer. These findings might provide a new insight into the possible mechanism for the toxicity of chromium ions at the level of the erythrocyte membrane.

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BIODISTRIBUTION OF SILVER NANOPARTICLES IN MALE WISTAR RATS AND THEIR POSSIBLE INTERACTIONS WITH PESTICIDE TOXICITY

Marcin Kruszewski1,2, Katarzyna Dziendzikowska3, Michal Oczkowski3, Agata Krawczyńska3, Joanna Gromadzka-Ostrowska3, Joanna Niedźwiecka2, Krzysztof Sawicki2, Malgorzata Cyranka2, Lucyna Kapka-Skrzypczak2,4

1 Independent Laboratory of Molecular Biology, Institute of Rural Health, Lublin, Poland
2Department of Public Health, University of Information Technology and Management, Rzeszów, Poland
3Centre for Radiobiology and Biological Dosimetry, Institute of Nuclear Chemistry and Technology, Warsaw, Poland
4 Department of Public Health, University of Information Technology and Management, Rzeszów, Poland
Correspondence: m.kruszewski@ichtj.waw.pl

Silver nanoparticles (AgNPs) are the most commonly used nanoparticles due to their antimicrobial properties. In this study we analyze (1) the effect of silver particle size on rat tissue distribution at different time points, (2) to determine the accumulation of AgNPs in potential rat target organs, (3) to examine the excretion of AgNPs by urine and feces. AgNPs (20 and 200 nm) were administered intravenously to male Wistar rats at a dose of 5mg kg -1 of body weight. Biological material was sampled 24 h, 7 and 28 days after injection. Using inductively coupled plasma-mass spectrometry (ICP-MS) it was observed that AgNPs translocated from the blood to the main organs and the concentration of silver in tissues was significantly higher in rats treated with 20nm AgNPs as compared with 200nm AgNPs. The highest concentration of silver was found in the liver after 24 h. After 7 days, a high level of silver was observed in the lungs and spleen. The silver concentration in the kidneys and brain increased during the experiment and reached the highest concentration after 28 days.

To further investigate a possible impact of AgNPs on environmental health their potential interactions with organophosphorus pesticide (chloropyrifos) was studied on cellular models reflecting different target organs (e.g. skin, lung, digestive tract and the brain). Determination of direct toxicity was measured by MTT test, whereas the compounds genotoxicity was determined by the comet assay. Obtained results suggest that NPs and CHP have a different cytotoxicity profile depending on concentration, time exposure and size of NPs and used cell line. Interestingly, binary mixtures of AgNPs and CHP showed synergistic toxic effect as compared to both compounds alone.

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BLOOD LEAD CONCENTRATION AND ITS RELATIONSHIP WITH GLOMERULAR FILTRATION IN A HOSPITAL WORKING POPULATION

Castillo Pérez C*, Diaz Díaz MA*, Martell Claros N**, Trasobares E*, Fuentes M*, Abad-Cardiel M**, García-Donaire JA**, González-Estecha M*.

**Laboratory Medicine Department. Trace Element Unit. **Hypertension Unit.*
Hospital Clínico San Carlos. Madrid (España)
Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC). Madrid (Spain).
Email: Montse@cmpx.net

Background. The level of lead considered to be toxic has declined over time, not only in children but also in adults. The aim of this study is to evaluate the association between blood lead concentrations and the estimated glomerular filtration rate (eGFR) adjusted by associated factors in a hospital working population.

Material and Methods. We recruited 395 employees (64 men and 331 women) with a mean age of 47.3 (SD: 10.9) years old. Blood lead concentration (µg/dL) was measured by atomic absorption spectrometry and thermal decomposition amalgamation. eGFR was assessed using the CDK-EPI formula.

Results. The median of blood lead was 1.7 µg/L (IQR:1.0-2.8) and the mean eGFR was 78.9 mL/min/l.73 m² (SD:11.9). Patients with an eGFR≤75 had a higher median of lead (2.3 µg/L; IQR: 1.4-3.5) than those with an eGFR>75 (1.3; IQR 0.8-2.5; p<0.001). Analyzing by lead quartiles, we observed that as blood lead concentrations increased, the percentages of subjects with an eGFR≤75 also increased. In the first quartile we found 18.8% of employees with an eGFR≤75, 40.8% in the second, 46.3% in the third and 54.3% in the fourth quartile (p<0.001). A logistic regression model was adjusted for hypertension, LDL-C, uric acid and age. We found that the subjects in the second, third and fourth lead quartiles were between 2 and 2.3 times more likely to have an eGFR≤75 (p=0.03) with respect to the first quartile.

However, when mercury and cadmium were introduced in the model, we observed a significant interaction between mercury and eGFR (p=0.043); this association is statistically significant only in the third lead quartile (OR=2.1 CI 95% 1.01-4.35;p=0.046).

Conclusions. Although blood lead concentrations have declined in recent years, we can still find an association between blood lead concentrations and eGFR. Environmental control of this pollutant continues to be indispensable.

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THE INTERCONNECTION BETWEEN ACCUMULATION OF TOXIC ELEMENTS IN HAIR AND DIFFERENT MORPHOLOGICAL STRUCTURES OF THE THYROID GLAND

Miroshnikov S.V. [1], Notova S.V. [1], Skalny A.V. [1,2], Alidzanova I.E. [1], Kiyaeva E.V. [1]

[1] *Orenburg State University, Orenburg, Russia*
[2] *Russian Society of Trace Elements in Medicine, Moscow, Russia*
E-mail: snotova@mail.ru

The study involved women aged from 35 to 50 years with colloid nodular (multinodular) euthyroid goiter. Euthyroid state of all patients was established by examination of TSH, free T_4 and T_3 in serum.

Hair samples and thyroid tissue (colloidal nodes and perinodular thyroid tissue) were used to study the trace element status of the body.

Samples of thyroid tissue were obtained during the execution of hemi- and/or total thyroidectomy. The elemental composition of samples was determined at the Center for Biotic Medicine (Moscow).The obtained concentrations of toxic elements in hair of patients were compared with optimal percentile interval. Content of the following elements was determined: Cd, Pb, Al, Sb, Bi, Hg. A tendency to a higher content of Cd in hair of the patients was shown. The level of Pb, Al, Sb, Bi, Hg in all examined hair was within the range of recommended values.

Besides the analysis of the average concentrations of toxic elements in biosubstrates, a correlation analysis of the data set was performed in the study. The relationship between content of specific elements in hair and tissues of the thyroid gland is very interesting in terms of non-invasive diagnostics. The correlation analysis have stated, that there is a significant direct correlation between content of Cd in the hair and content of Cd in perinodular thyroid tissue($r = 0.65$). For other toxic elements no correlation relationships were found.

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DI(2-ETHYLHEXYL)PHTHALATE-INDUCED TESTICULAR, HEPATIC, RENAL AND THYROIDAL OXIDATIVE STRESS AND EFFECTS OF DIFFERENT SELENIUM STATUS

Pinar Erkekoglu[1], Belma Koçer Giray[1], Walid Rachidi[2], Isabelle Hininger-Favier[3], Dilara Zeybek[4], Esin Aşan[4], Anne-Marie Roussel[3], Alain Favier[2], Filiz Hincal[1]

[1] *Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara Turkey*
[2] *CEA Grenoble, INAC/SCIB/LAN, 17 Rue des Martyrs, Grenoble Cedex 9, France*
[3] *LBFA, Universite Joseph Fourier, Grenoble, France*
[4] *Hacettepe University, Faculty of Medicine, Histology and Embryology Department, Ankara, Turkey;*
**Correspondence: fhincal@hacettepe.edu.tr; bgiray@hacettepe.edu.tr*

Phthalates are most abundantly used plasticizers. Different mechanisms were suggested for their toxicity and an ever increasing possibility is the induction of oxidative stress. Selenium (Se) is one of the key essential trace elements. This study was designed to investigate the effects of DEHP on oxidant/antioxidant parameters in different rat organs and to evaluate the possible changes by different Se status. In 3-weeks-old Sprague–Dawley rats, Se deficiency was produced by feeding them <0.05 mg Se/kg diet, and Se supplementation group was on 1 mg Se/kg diet for 5 weeks. DEHP-treated groups received 1000 mg/kg dose by gavage during the last 10 days of the feeding period. Activities of antioxidant selenoenzymes, and other antioxidant enzymes; total glutathione (GSH) concentrations and thiobarbituric acid reactive substance (TBARS) levels were measured. Testicular and hepatic apoptosis were measured and histopathological evaluations were also performed. DEHP was found to induce oxidative stress in different rat organs, as evidenced by decreases in GSH concentrations (~60% in testis, ~15% in liver, ~20% in kidney) and increases in TBARS levels (~80% in testis, ~40% in liver, ~30% in kidney, and ~35% in thyroid). Moreover, DEHP induced histopathological changes in both testis and liver whereas apoptosis was only induced in testis. Se supplementation reduced the oxidative stress produced by DEHP whereas Se deficiency ameliorated effects of DEHP. These findings emphasized the critical role of Se as an effective redox regulator and the importance of Se status in protecting rat tissues from the oxidant stressor activity of DEHP.

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STUDY OF BIOMARKERS OF THE EFFECTS OF LEAD ON HEME BIOSYNTHESIS

José Manuel González-López (a), Elena María González-Romarís (b), Isabel Idoate (c) and Jesús Fernando Escanero (d).

(a). Clinical Biochemistry Service. Miguel Servet University Hospital, Zaragoza, Spain.
(b). Clinical Laboratory. Galician Health Service, Santiago de Compostela, Spain.
(c). Clinical Laboratory. Navarra Hospital Complex, Pamplona, Spain.
(d). Department of Pharmacology and Physiology. University of Zaragoza, Faculty of Medicine, Zaragoza, Spain. escanero@unizar.es

Lead (Pb) causes the inhibition of the enzymes ALA-Dehydratase (ALA-D) in cytosol and coproporphyrinogen oxydase and of ferrochelatase in mitochondria. Consequently, a decrease is produced of the activity of ALA-D and an increase of protoporphyrin IX (Proto IX) in erythrocytes. Furthermore, the urinary excretion of δ-aminolevulinic acid (5-ALA) y coproporphyrin III is increased.

This study analyses and compares the values of lead in blood and of ALA-D and Proto IX in erythrocytes in two samples of healthy people studied with an interval of 20 years, to verify the effect of the improvements in environmental and work conditions as well as in a group of patients with lead poisoning, in whom 5-ALA and coproporphyrins were also studied in urine.

The study was retrospective.

a). Groups: Control group in adults (Year 1990: ALA-D, Proto IX, Proto IX/g Hb and Pb in blood (PbB) of 165 cases; 5-ALA, and coproporphyrins and Pb in urine (PbU) of 325 cases. Year 2012: PbB, in blood of 156 people and ALA-D and Proto IX, in blood of de 1336 cases). Study group (lead poisoning): 39 cases.

b). Analytic methods used:

ALA-D and free Proto IX in blood by means of kits of Immuno Pharmacology Research (IPR). 5-ALA by means of the Bio-Rad kit. Porphyrins in urine (uro- and coproporphyrins) applying a fluorimetric method. PbB by means of FAAS, in 1990; and by means of ETAAS in graphite furnace with Zeeman correction, in the 2012 review.

The results (x±SD) of the adult control group (male and female), were: Year 1990, blood: PbB (µg/dL, 16.54±5.49), ALA-D (U/mL, 46.81±15.45), Proto IX (µg/dL, 28.13±10.87), Proto IX/g Hb (1.96±0.82); urine: PbU (µg/24h, 40.96±35.01), 5-ALA (mg/24h, 2.79±1.28), COPRO- (µg/24h, 90.15±52.88). Year 2012, blood: PbB (µg/dL, 2.94±2.02) and ALA-D: (U/mL, 58.58±36.11), Proto IX (20.14±11.40), Proto IX/g Hb (1.31±0.55).

Comparing the means of 1990 and 2012, statistically significant differences were observed ($p<0.001$) in PbB, ALA-D, Proto IX and Proto IX/g Hb.

In the lead poisoning group there was a significant increase ($p<0.001$) of PbB and PbU, a decrease ($p<0.001$) of ALA-D and an increase ($p<0.001$) of Proto IX , Proto IX/g Hb, 5-ALA and COPRO-.

In summary: 1. PbB (and ALA-D, Proto IX and Proto IX/g Hb) of the control group decreased significantly in 2012, which indicates lower levels of environmental and work contamination, probably due to stricter norms. 2. The biomarkers studied are very efficient in the diagnosis and developmental control of lead poisoning.

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EFFECT OF COMBINATION THERAPY AGAINST ALUMINIUM INTOXICATION

Sadhana Shrivastava and Sangeeta Shukla

UNESCO Satellite center of Trace Element Research &Reproductive Biology and Toxicology Laboratory, School of Studies in Z oology, Jiwaji University, Gwalior-474011, India dr_sadhana59@rediffmail.com

Aluminum is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere. It is a potent neurotoxin and has been associated with Alzheimer's disease causality for decades. A number of studies support the leaching of Al and experts now warn that with the increased use of it in packaging of foodstuffs the potential source of contamination is higher. It is toxic metal to living organisms, including human beings, was discovered a long time ago. It can reach and accumulate in almost every organ in the human body, the central nervous system is a particular target. The involvement of oxidative stress, membrane biophysics alterations, deregulation of cell signaling, and the impairment of neurotransmission as key aspects involved Al. It is sometimes a competitive inhibitor of several essential elements of similar characteristics, such as Mg, Ca, and Fe. So in the present study therapy with Se along with HEDTA was given. The aim of the study was to demonstrate the protective effect of HEDTA administration against aluminium-induced dysfunction in rats. Aluminium Nitrate (27 mg/kg of Al, i.p.for 60 days) was administered to rats, treated with HEDTA along with Se 7 days. Significant rise were observed the activities of serum alkaline phosphatase, AST and ALT whereas serum protein and albumin was found to be decline after toxicant exposure. The concentration of Al increased in liver and serum causing significant increase in the blood urea nitrogen, creatinine and urea level. Secondary products of lipid peroxidation that is MDA levels were estimated by measuring the TBARS levels. Our results indicate that TBARS levels was significantly higher and total glutathione content were significantly lower during toxicity. The decrease in activity of various enzymes viz. succinic dehydrogenase, adenosine triphosphatase and glucose-6-phosphatase were noted Combination therapy showed over all improvement in all the biochemical changes. Al exposure increased collagen fibers and degenerated cristae of mitochondria in the liver. Well formed mitochondria, endoplasmic reticulum and nucleus were seen after therapy of HEDTA+Se. Electron microscopical changes in liver and kidney were also recouped with the therapy. In conclusion, HEDTA+Se has protective effects against aluminium-induced dysfunction and oxidative damage.

15. Environment

2-104

STUDY OF SOME PLANTS BY USING OF INSTRUMENTAL NEUTRON ACTIVATION AN ALYSIS METHOD IN UZBEKISTAN

S.Bakiev, B.Kuldjanov, N.Osinskaya, J.Rakhmanov, Y.Akhmedov

Institute of Nuclear Physics, Tashkent, Uzbekistan

Although many studies dedicated to investigation of elemental contents of plants there are not much data for area of Republic of Uzbekistan. In this regard any additional appropriate information is a subject of interest. Many trace elements play essential role for human organism in general as well as in cure of diseases associated with microelements deficiency or excess. Since contents of trace elements in plants are very small, a sensitive analytical method needed to obtain of precise data. Instrumental neutron activation analysis method was used to analyze about 40 elements in different plant samples collected mostly around Tashkent city in Uzbekistan. The samples were irradiated with neutrons in a nuclear reactor and the induced activities were counted by ray spectrometry using a high purity Germanium detector with high resolution. Total quantity of plant types was about 200 with total quantity of samples measured is about 400. The plant types included vegetable food products, seeds, medicinal herbs and some of folk remedies.The elemental contents and their dependencies for plant types, area of origin and plant parts were obtained. The maximal concentrations for more than 10 vital elements were determined. The possibilities in use of data obtained for nutrition at microelements deficiencies are discussed.

2-105

RADON AND PHOSPHATE FERTILIZERS PRODUCTION

Kist A.A., Vasidov A., Danilova E.A.

Institute of Nuclear Physics, Uzbekistan Academy of Sciences, Tashkent, Uzbekistan
danilova@inp.uz

Determining high concentrations of uranium and thorium in the phosphate rocks and taken in consideration of the rocks dissolution by sulfuric acid we assumed probability of the radon appearance in the atmosphere. Radon concentrations were measured in various rooms in the Uzbekistan phosphate fertilizers factory, in vicinity of the factory and in the flats of the city in which the factory is situated. For comparison radon was also determined in the city (Tashkent) in which there are not factories working with phosphate rocks.Radon measurements were carried out by SSNTD (solid state nuclear track detectors) technique. Detector used: CR-39 (polyallyl-diglycol carbonate).

Geometry of the device: A piece of the CR-39 plastic track detector of 2.5×2.5 cm2 was put on the bottom of the "cup detector sampler". The cup was made of polyethylene and had a shape of truncated cone with an open end. After putting the detector, the open end was closed by a paper filter in order to prevent the detector from dust and other solid particulates and also from the radon progeny plate-out effect on the detector surface. Thus, the device detects only alpha particles from a radon gas and those daughters, which are produced inside the cup.

Geometry of experiment: The detector samplers were suspended on a ceiling on the distance of ~200 cm from the floor. The detectors were exposed for 15 – 30 days.

Chemical treatment: The exposed plastics were chemically etched in the 6N NaOH solution at 700 C, for 6 hours, so that the tracks can be observed in an optical microscope at 100 – 500 magnification.

To determine the calibration factor of the SSNTD device the radon concentration measurements were carried out by both techniques (with SSNTD and intercalibrated Alpha GUARD) in the same rooms.

Radon concentrations in the factory were found significantly higher than safe limits given by ICRP. In the flats in the studied city concentration of radon was found also higher than in "clean" city but lower than allowed limit.

Data for the studied factory and city say that, probably, the radon may be a reason for increased respiratory system diseases and lung cancer. It is necessary to note that, at least in Uzbekistan, radon is not monitored on working places of the phosphate fertilizers factories.

2-106

ANALYSIS OF GADOLINIUM IN ALGAE AND WASTE HOSPITAL WATER

Anna Krejčová1, Jakub Návesník1, Jana Zelenková2, Tomáš Černohorský1, Miloslav Pouzar1

1 Institute of Environmental and Chemical Engineering, Faculty of Chemical Technology, University of Pardubice, Studentská 95, 532 10 Pardubice, Czech Republic
2 Department of Hydrodynamics, Academy of Science, Pod Paťankou 30/5, 160 00 Praha, Czech Republic

Contrast mediums based on gadolinium compounds have been utilized for an imaging using magnetic resonance since 1988. These Gd-complexes show great stability and inertness inside human body and therefore are excreted in an unchanged form. Excreted Gd compounds follow a regular way of waste water disposal and even despite this process; Gd persists and continues to surface waters. An increased concentration of Gd of an anthropogenic origin in surface waters known as "the positive gadolinium anomaly" have been studied recently. Anthropogenic Gd enters water ecosystems and can accumulate in river plants, organisms and sediments. Natural food chain could be the way of possible health risk for humans. With regards to widespread utilizing of Gd based contrast mediums for magnetic resonance, a monitoring and elimination of anthropogenic gadolinium in natural ecosystems appears to be necessary.

In our study, a possible use of time of flight inductively coupled plasma mass spectrometry for determination of lanthanides, especially Gd, in waste water was investigated. Examined waste waters were provided by two easts–Czech hospitals (College hospital in Hradec Králové and Regional hospital of Pardubice). Obtained data proved the positive gadolinium anomaly. Second part of this study was dedicated to the bioaccumulation experiment. Algae of family Chlorella was cultivated in Gd mediums. Bioaccumulation factors were assessed after one months of cultivation. We faced a contamination problem during the bioaccumulation experiment as well problems connected with a small sample preparation.

2-107

REMOVEMENT OF Sr FROM SOIL USING SILICIC ACID SOLUTION

Takahashi M.

Miho Tanaka1 and Kazuya Takahashi2 1Graduate

School of Marine Science and Technology, Tokyo Univ. of Marine Sci. and Tech.mihotnk@kaiyodai.ac.jp, 2Riken, Kazuyat@riken.jp

1. The disaster of the East Japan Earth Quake brought the accident of the Fukushima Electric Power Plant. 137Cs(t1/2 30.17 y) and 90Sr(t1/2 28.79 y) fell on the surface of the ground.

In Japan, treatment of radioactive contamination is a very important problem. The contaminated soils were gathered and are still located in corners of school grounds. To develop a method of removal, some points should be resolved. 1) no change to the quality of original soil 2) treatment of removal scheme without any artificial pollution 2. The humic and fulvic acids in leaf molds were extracted by 0.1M NaOH, and arranged with 0.1M HNO_3 to become pH 5.766 (i.e. the standard solution of mold). The solution, which was obtained from "water silica"（nutrients for rice）and pure water, was a 2.3mM Si concentration without any metal ions measured by ICP-MS. The chemical species were identified by two Mass Spectrometries: FAB and ESI. 3. Sr Characterization Silicic acid ($Si(OH)_4$) could easily be made complex with Sr^{2+}. When a water silica solution and 0.1M Sr was shaken, the peak of $[SiO_3(OH)Sr]^-$ was observed with both spectra. And 0.1M Ca was added to this solution after a day. $[SiO_3(OH)Sr]^-$ was not changed to $[SiO_3(OH)Ca]^-$. On the other hand, $[SiO_3(OH)Sr]^-$ in the water silica solution and 0.1M Sr was stable even if a standard solution of mold was added. $[SiO_3(OH)Sr]^-$ is reported to be stable in our previous study. And this result indicates that Sr could be removed from soil and mold by adding silicic acid. 4. Cs Characterization Silicic acid could easily be made complex with Na^+ and K^+. Low intensity $[SiO_2(OH)_2Cs]^-$ was recognized in a standard solution of mold and 0.1M Cs. In the standard solution of mold and 0.1M Cs, and adding Water Silica after 1day, $[SiO_2(OH)_2Cs]^-$ was observed having a low peak. A small amount of $[SiO_2(OH)_2Cs]^-$could be removed from the soil, but more study will be required for removal of large amounts of Cs ions.

2-108

NEW METHOD FOR COMPREHENSIVE DETECTION OF TRACE ELEMENTS IN ENVIRONMENTAL OR BIOCHEMICAL MATERIALS USING AN ELECTRON-CYCLOTRON-RESONANCE ION-SOURCE MASS SPECTROMETER

Tatsuya Urabe[1], Masanori Kidera[1], Michiko Kitagawa[1], Tsuyoshi Nagamatsu[1,2], Masayoshi Toda[3], and Kazuya Takahashi[1]

[1] *RIKEN, kazuyat@riken.jp*
[2] *Tokyo University of Science*
[3] *Tokyo University of Marine Science and Technology*

We have developed a detection technology for trace elements, including their chemical complexes, based on an element analytical system using an electron-cyclotron-resonance ion source (ECRIS). An electron exhibits cyclotron motion in a magnetic field and is accelerated resonantly upon applying a high frequency. This phenomenon is called electron cyclotron resonance (ECR). ECR has been used as the ion source in accelerator facilities. We have focused our attention on the stability and high ionization efficiency of ECRIS (ECR Ion Source) and we employed ECRIS to customize a mass spectrometer to realize ECRIS mass spectroscopy (ECRIS-MS).

Using ECRIS-MS, the following three applications were carried out. (1) Isotopic analyses of trace elements in geochemical or environmental materials. (2) Trace analyses of metal ions in biological or medical samples such as blood. (3) Monitoring of hazardous chemicals such as chemical warfare agents (CWAs) in the atmosphere. In particular, for applications (1) and (2), sputtering and laser ablation techniques has been also employed to assist sample introduction into the ion source without any complicated chemical procedures. These applications are expected to contribute to the environmental and biochemical research fields. For the monitoring of hazardous chemicals (3), a vapor sample is introduced directly into the ion source, and the molecular material was decomposed into elements using the electron cyclotron resonance plasma and ionized. The type of chemical warfare agent, specifically whether it was a nerve agent, blister agent, blood agent, or choking agent, could be determined by measuring the quantities of the elemental ions or fragmented molecular ions using mass spectrometry. We will present details of the potential of ECRIS-MS for the detection and identification of trace elements in various environmental or biochemical samples.

2-109

STUDIES ON AS AND SE UPTAKE BY MUNG BEAN

Ajoy Mandal1, Kaustab Ghosh1, Moumita Maiti2, Susanta Lahiri1*

1 Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata-700064, India
2 Department of Physics, Indian Institute of Technology Roorkee, Roorkee-247667, India

Mung bean (Vigna radiata) is a common food grain in Indian subcontinent. They grow rapidly and the effect of pollution can be evaluated in a short period of time. We have examined the efficiency of uptake of As and Se from the contaminated sand in different parts of Mung beans cultivated in the laboratory condition. The data may give an insight towards the bio-magnification behaviour of As and Se to human body.

Healthy Mung bean seeds were sown in 50 g sand containing spiked Hoagland's nutrient solution. Four replica were prepared for each concentration of As(III) , Se(IV) and As(III) + Se(IV) separately. The seedlings were removed and washed with distilled water. Then plants were divided into two parts, namely the root and shoot. Dried samples were microwave digested with HNO_3/H_2O_2 mixtures. The total amounts of As and Se absorbed under different conditions by these parts were measured by ICPOES.

It was observed that seedlings were not germinated at all by addition of 15 mL 15 ppm As solution to the sand bed. Even addition of 1.5 ppm As solution resulted wrinkling of the leaves. Whereas normal growth of seedlings were observed till addition of 9 ppm Se, and upto 15 ppm concentration of Se, only slight hindrance in growth was observed. Therefore we took solutions of As from 15 ppb to 1.5 ppm; while 1 ppm to 15 ppm Se solutions were taken. No As uptake was observed upto 150 ppb As solution in the sand bed. However addition of 0.5 ppm to 1.5 ppm solution shows uptake of As in Mung beans and the concentration of As was magnified upto the level of 0.5 to 4 ppm. Roots showed relatively higher uptake. Similarly a direct relation between uptake of Se and concentration of Se solution added to the sand was observed. The relative uptake of Se in shoot was less than that of root. Maximum Se concentration in root was ~ 215 ppm. Further, Se accumulation was reduced in presence of minute amount of As while As uptake was higher in presence of Se.

* To whom correspondence would be addressed: Fax: +913323374637, E mail: susanta.lahiri@saha.ac.in

2-110

INTESTINAL TAPEWORMS INFLUENCE TRACE ELEMENT CONCENTRATIONS IN FISH: THE CASE OF CLESTOBOTHRIUM CRASSICEPS IN THE EUROPEAN HAKE (MERLUCCIUS MERLUCCIUS) FROM THE MEDITERRANEAN COAST OF GIRONA

J Torres 1,2*, C Eira 3, J Miquel 1,2, D. Ferrer 4, S Vila 4, E Delgado 4 & M Casadevall 4

1 Department of Sanitary Microbiology and Parasitology, Pharmacy Faculty, Laboratory of Parasitology, University of Barcelona, Joan XXIII Avenue, 08028, Barcelona, Spain. E-mail: jtorres@ub.edu
2 Institut de Recerca de la Biodiversitat, University of Barcelona, Av. Diagonal, 645, 08028 Barcelona
3 CESAM and Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal.
4 Departament de Ciències Ambientals, Universitat de Girona. Campus Montilivi s/n., 17071 Girona

Predator marine fishes may bioaccumulate high levels of trace elements. The harmful effects of Cd, Pb, and Hg on human health motivated the European Commission to regulate their maximum levels in fish. In Catalonia the European hake (M. merluccius) has great economic importance and it is quite abundantly consumed (about 5-6 Kg/person/year) constituting an important part of the human daily intake of trace elements. The capacity of fish parasites to bioaccumulate trace elements is currently admitted, thus parasitized individuals tend to present lower trace element levels. This feature has been widely discussed from an environmental point of view, considering parasites as sentinel organisms. However, the influence of parasites has never been taken into account when evaluating potentially toxic element concentrations in edible fishes. In this context, we report the effect of Clestobothrium crassiceps on trace element concentrations in hakes caught in the Mediterranean Sea. Thirty hake specimens (15 infested by C. crassiceps and 15 without the cestode) purchased in the port of Roses (between 2010 and 2011) were analysed. Edible muscle samples as well as cestodes (100 - 200 mg wet weight) were digested with HNO_3 and H_2O_2 at 90 ºC during 12 hours. Samples were analysed by ICP-MS. The analytical procedure was checked by using reference materials (DOLT-3 and DORM-2) and blanks. Cadmium values in the edible muscle of both subsamples were always below 1 ppb (ng/g w.w.). The mean muscular lead concentration of non-infested hakes was 6 ppb whereas in infested specimens the mean Pb concentration was 4 ppb. Total mercury concentration in the edible muscular part was 610 (in non-parasitized hakes) and 510 ppb (hakes with Clestobothrium). In addition, the concentration of muscular selenium, element that seems to have a protective effect against human mercury toxicity is higher in infested fishes (480 ppb versus 410 ppb). Study partially financed by the Spanish project CTM2009-08602

2-111

TRACE ELEMENT CONCENTRATIONS IN PARANOPLOCEPHALA OMPHALODES (CESTODA) IN COMPARISON TO ITS HOST, *MICROTUS ARVALIS* IN BELARUS

J Torres[1,2*], C Eira[3], J Miquel[1,2], C Feliu[1,2] & V. Shimalov[4]

[1]*Department of Sanitary Microbiology and Parasitology, Pharmacy Faculty, Laboratory of Parasitology, University of Barcelona, Joan XXIII Avenue, 08028, Barcelona, Spain. E-mail: jtorres@ub.edu*
[2]*Institut de Recerca de la Biodiversitat, University of Barcelona, Av. Diagonal, 645, 08028 Barcelona*
[3]*CESAM and Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal.*
[4]*Brest State University, Boulevard of Cosmonauts, 21, 224665 Brest, Belarus*

Over the last decades parasites have been attracting attention as potential indicators of environmental quality in aquatic ecosystems. More recently, some attention has also been drawn to parasites as potential environmental indicators in terrestrial ecosystems, either in theoretically polluted areas or in areas where major ecological accidents have occurred. Contrarily, insufficient attention is devoted to areas where environmental quality is acceptable. However, it should be taken into account that long-term pollutant activities may disturb the more pristine ecosystems. Thirty-four *Microtus arvalis* specimens parasitized by *Paranoplocephala omphalodes* from the Brest region (south-west Belarus) caught during 2008 were analysed. Tissue samples (liver, kidney and muscle) as well as cestodes (150 mg wet weight) were digested with HNO_3 and H_2O_2 at 90 ºC during 12 hours. Samples were analysed by ICP-MS. The analytical procedure was checked by using reference materials (DOLT-3 and DORM-2) and blanks. Bioaccumulation factors (BFs) can be defined as the ratio of the element concentration in the parasites to that in different host tissues. As expected high cadmium values were observed in kidney with an average value of 79.7 ppb while the muscular level was 12.6 ppb. Similar trends occurred with copper, lead and mercury in kidney, respectively 6.1 ppm, 85.1 ppb and 12.9 ppb and also in muscle, respectively 3.1 ppm, 17.8 ppb and 5.4 ppb. Mean levels of Pb and Hg in *P. omphalodes* specimens were 627.4 and 42.1 ppb, respectively. All intestinal cestodes absorb nutrients across their tegument from the host intestinal lumen. Although more field essays are necessary, considering the BFs obtained in the present study the system *P. omphalodes* / *M. arvalis* can be proposed as a promising bioindicator system to evaluate Pb and Hg pollution in terrestrial environments.

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2-112

POLYSACCHARIDES OF DIFFERENT SPECIES OF BROWN AND RED ALGAE GROWING ON THE LEBANESE COAST: ISOLATION, STRUCTURAL FEATURES WITH ANTIOXIDANT, ANTICOAGULANT AND ANTITUMOR ACTIVITIES

Carine Sebaaly, Samar Rachidi and Hussein Kanaan*

Laboratory of Biotechnology of Natural Substances and Health Product, Faculty of Pharmacy, Lebanese University, Beirut, Lebanon
E-mail: Hkanaan@ul.edu.lb, Carine.sebaaly@yahoo.com, srachidi60@hotmail.com

In addition to their high nutritional value, brown and red algae are of great interest as a source of therapeutic substances. However, despite their abundance on the Lebanese coast seaweeds have never been studied before. Therefore, we attempted to isolate the polysaccharides from the red algae *Pterocladia* and from different species of brown algae *Dictyopteris polypodioides*, *Sargassum* and *Padina pavonica*. The extraction yields were 2.7% for sulfated galactans and 11.5% for carrageenans isolated from the red seaweed. From the brown algae, the extraction yields of polysaccharides of *Padina* were 5% and 10.5% for alginate, 2.25% and 3.35% for F.L.M complex (Fucoidan, Laminaran, Mannuronan), in April and July respectively. From *Dictyopteris* we obtained 4.6% and 6.25% for alginate, 3.75% and 5.8% of FLM complex in May and July respectively. As well, 16.3 % of alginates were extracted from *Sragassum*. In order to elucidate their structures, H^1 NMR spectroscopy was performed as well as Infra-Red spectroscopy which allowed us to reveal their functional groups. Moreover, the analysis of minerals and trace elements by means of atomic spectroscopy showed large amounts of potassium, calcium, silicon, magnesium and iron in all these studied algae. The fatty acid composition of these algae was determined using gas chromatography and showed that the palmitic acid was the major fatty acid in these samples. Furthermore, fucoidans isolated from three different species of brown algae exhibit antitumor activity against RPMI-7951 human melanoma cells. The anticoagulant activity of polysaccharides was investigated by activated partial thromboplastin time (APTT) clotting assay and the antioxidant activity was studied by electrolysis method. It has been shown that FLM and Mannuronan fractions have a significant antioxidant and anticoagulant activities. Sulfated galactans exhibit more pronounced antioxidant and anticoagulant effects than carrageenans. Further investigations are imperative in order to develop new molecules based on these polysaccharides to be used as drugs or for healthcare in general.

2-113

LEVELS OF HEAVY METALS IN VARIOUS PROCESSES OF WASTEWATER TREATMENTS AND THEIR ROLES ON PATHOGENIC MICROORGANISMS

Ching-Wen Chang1, Kai-Wen Ming1, Yaw-Huei Hwang2, Cheng-Chi Liao2

1 Institute of Environmental Health, National Taiwan University, Rm 740, No.17, Xu-Zhou Rd., Taipei, 10055, Taiwan, R.O.C. E-mail (C-WChang):chingwenchang@ntu.edu.tw
2 Institute of Occupational Medicine and Industrial Hygiene, National Taiwan University

Metallic elements may be present in sewage water, which could be beneficial or detrimental to Acanthamoeba spp. and Hartmannella vermiformis, known as free-living amoebae (FLA) and pathogens of meningoencephalitis in humans. The aims of this study were to determine the effects of wastewater treatments and disinfection processing on 15 heavy metals (Be, Al, V, Cr, Mn, Co, Ni, Cu, As

Water sample was taken from the influent sewage prior to any treatment and the effluent sewage from the tanks of secondary clarification and chlorination, respectively. Heavy metals and FLA were quantified by inductively coupled plasma mass spectroscopy and real-time PCR targeting on 18S rDNA, respectively. Total bacterial count was determined on R2A agar. Residual free chlorine, pH, water temperature, hardness, conductivity and turbidity were measured in situ whereas suspended solids and dissolved organic compounds were analyzed in laboratory.

All 15 heavy metals were detected in influent sewage. Mn, Al, Zn and Ba were ranked as the highest constituents with a mean level greater than 5 μg/L (i.e. 51.8, 30.4, 14.6 and 8.5 μg/L, respectively), followed by Cu (3.5), Ni (2.7), Cr (2.3), As (1.4), V (0.8), Pb (0.4), Th (0.4), Sb (0.2), Co (0.2), Cd (0.06) and Be (0.05). Following the sedimentation, biodegradation and clarification, 87.3% of Al and 38.2% of Mn was removed. Compared to the level in influent sewage, Al, Cr, Mn, Co, Cu, Pb and Sb were all significantly decreased in treated water after chlorination (all $P< 0.05$). In contrast, Zn, V, As and Sb were not changed.

Spearman correlation analyses showed the conductivity and Al, Cr and Mn were positively correlated with the level of Acanthamoeba (all $P< 0.05$, $r = 0.71$, 0.77, 0.67 and 0.66, respectively). In contrast, the abundance of H. vermiformis was negatively associated with the levels of Co, Ni and As (all $P <0.05$, $r = -0.65$, -0.64 and -0.86, respectively) but positively related to pH and hardness (both $P <0.05$).

This study illustrates that the sedimentation, biodegradation and clarification may significantly reduce most of heavy metals. Moreover, heavy metals are important factors influencing FLA abundance in sewage; however, their effects could be positive or negative, depending on metal type and FLA genus.

2-114

ASSESSMENT OF THE HEAVY METALS IN THE FOOD FROM ROMANIA, 2009 - 2011

Carmen Hura, B.A.Hura, C. Pancu

INPH/CRPH Iasi,
Str.v.Babes nr.14 Iasi, Romania carmen _ hura@yahoo.com

Exposure to heavy metals is an important problem of environmental toxicology. Most of these metals are toxic to humans, animals and plants. Man, being at the top of the food chain, is at great risk of suffering from health hazards associated with toxic metals because of bioaccumulation. The aim of this study was the evaluation of the heavy metals contents in the food from Romania area.

The study presents the results obtained in 2009- 2011 of some metals [Pb, Cd] in the food, 1869 samples: meat (469 samples), vegetables (750 samples), panification products (283 samples), juice (162 samples), diets (205 samples), in Romania. Trace elements concentrations were analyzed by atomic absorption spectrophotometry.

In all analysed samples these metals were found. Generally, a wide variation between individual samples was observed.

Meat: The mean metals levels in the meat products varied between 0.07 mg/kg Cd and 0.08 mg/kg Pb.

Vegetables: The mean metals levels in the vegetables varied between 0.02 mg/kg Cd and 0.07 mg/kg Pb.

Panification products: The results of the investigations showed a variation of heavy metals between 0.02 mg/kg Cd and 0.06 mg/kg Pb.

Diets: The mean metals levels in the diets varied between 0.03 mg/kg Cd and 0.1 mg/kg Pb.

Determinations of these chemical contaminants in food are important in environmental monitoring for the prevention, control and reduction of pollution as well as for occupational health and epidemiological studies.

2-115

MERCURY IN CANNED TUNA IN SPAIN. IS LIGHT TUNA REALLY LIGHT?

González-Estecha M[1*], Martínez MJ[2], Guillén JJ[3], Bodas A[4]*, Fuentes M[5]*, Ordóñez JM[6], Martell N[7]*

[1] *Laboratory Medicine Department. Trace Element Unit.* [2]*Universidad Politécnica de Cartagena Spain.*
[3] *Public Health Department Murcia (Spain).* [4]*Pediatric Department.* [5] *Research Unit.* [6]*Consejería de Sanidad Madrid (Spain).* [7]*Hypertension Unit*
* *Hospital Clínico San Carlos. Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC). Madrid (Spain).*
Email: montse@cmpx.net

Background. The few studies done in Spain have shown high blood mercury (Hg) levels due to the high consumption of fish by the population. Some studies have stated that the most-consumed fish in Spain is canned tuna. Different Spanish organisms and regulatory agencies consider that it is safe to consume canned tuna as it supposedly has a low mercury content, particularly in so-called light tuna. However, in Spain light tuna is mainly Yellowfin and Bigeye tuna, while in other countries it is mainly Skipjack.

Material and Methods. We analyzed 36 cans of the most popular brands in Spain. We examined the influence of different brands, price, type of tuna and packaging medium (water, olive oil, sunflower seed oil or marinade). Mercury concentrations (mg/kg) were measured by atomic absorption spectrometry and thermal decomposition amalgamation.

Results. The medians observed were (mg/kg): Light tuna:0.314 IQR 0.205-0.594; white tuna:0.338 IQR 0.276-0.558; skipjack:0.311 IQR 0.299-0.322; frigate tuna:0.219 IQR 0.182-0.257; mackerel:0.042 IQR 0.029-0.074. We found statistically significant differences between white tuna, light tuna and mackerel (p=0.004); light tuna and mackerel (p=0.002) and white tuna and mackerel (p=0.006). However, we found no differences between white tuna and light tuna, or between brands, packaging medium or price. The limit of 0.500 mg/kg of mercury in canned tuna was exceeded by the following percentages of the cans: 33.3% of light tuna, 16.7% of white tuna, and 0% of Skipjack, frigate tuna and mackerel.

Conclusions. The mercury content of the cans of Spanish light tuna that were analyzed was variable and high. There were no difference with respect to price, brand or packaging medium. The results of this study indicate that stricter regulation of Hg in canned tuna is necessary. Until then, it is safer to recommend that vulnerable populations such as children and pregnant women consume canned mackerel, which has a markedly lower mercury content.

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CONCENTRATION OF MAGNESIUM, CALCIUM, ZINC, COPPER AND MANGANESE IN BEE POLLEN FROM POLAND

Agnieszka Wojtanowska-Krośniak[1)], Jolanta Kaleta[2)], Miroslaw Krośniak[1)], Malgorzata Wilusz[2)], Pawel Zagrodzki[1,3)]

1) Dept. of Food Chemistry and Nutrition, Medical College, Jagiellonian University, Kraków, Poland, a.wojtanowska-krosniak@uj.edu.pl, mfkrosni@cyf-kr.edu.pl
2) The Apiarian and Pharmaceutical Company "APIPOL-FARMA" Ltd, Myślenice, Poland, jolanta@apipol.com.pl, malgorzataw@apipol.com.pl
3) H. Niewodniczański Institute of Nuclear Physic, Dept. of Nuclear Physical Chemistry, Kraków, Poland, Pawel.Zagrodzki@ifj.edu.pl

The bee pollen is the flower pollen collected by bees. It's the main protein source for bees. Bee pollen contains many biologically active compounds like proteins, carbohydrates, flavonoids, vitamins, carotenoids, low molecular weight organic acids, fatty acids including unsaturated fatty acids, free aminoacids, nucleic acids, nucleosides and micro- and macro- minerals. For this reason, bee pollen is used as a diet supplement.

The main purpose of the research was to assess the levels of macro- and microelements like potassium, magnesium, copper, calcium, zinc and manganese in samples of Polish origins, and then compare them against the Recommended Daily Allowances (RDA) of these minerals.

All samples of bee pollens, after mineralization (digestion in microwave oven with nitric acid) were studied using flame AAS method. Despite the different regions and time periods of collecting, obtained results were similar. Maximal difference between minimum and maximum concentrations was observed in case of manganese- about times, and minimal difference was observed in case of calcium - less than two times. The results for Mg were from 0.917 mg/g to 3.724 mg/g, x_{mean}= 1.312 mg/g, for Ca from 0.906 mg/g to 1.386 mg/g, x_{min}=1.109, for Cu from 6.95 μg/g to 13.34 μg/g x_{mean}= 8.93 μg/g, for Zn from 26.80 μg/g to 59.66 μg/g, x_{mean}= 43.14 μg/g, and for Mn from 12.02 μg/g to 29.60 μg/g x_{mean}= 20.08 μg/g.

We observed negative correlation between magnesium vs. copper r= -0.712, p<0.05, and second one between magnesium and zinc r= -0.735, p<0.05. Positive correlation was observed only for copper and zinc r= 0.838, p<0.05.

Results of the research showed that bee pollen is reach source of copper and manganese and recommended dose of bee pollen (30 g-45 g per day) correspond with 26%-39% RDA of copper and 30%-45% of manganese.

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PREPARATION OF SILVER DOPED HYDROXYAPATITE THIN FILMS WITH ENVIRONMENTAL APPLICATIONS

S.L. Iconaru[1,2], E. Andronescu[3], C. Ciobanu[1], R.V. Ghita[1], A.M. Prodan[4,5], P. Chapon[6], P. Le Coustumer[7, 8], A. Costescu[2], D. Predoi[1*]

1 *National Institute for Physics of Materials, P.O. Box MG 07, Bucharest, Magurele, Romania,*
**Telephone: +40213690185, Fax: +40213690177, dpredoi@gmail.com*
2 *Faculty of Physics, University of Bucharest, 405 Atomistilor, CP MG-1, 077125 Magurele, Romania*
3 *University Politehnica Of Bucharest, Faculty Of Applied Chemistry And Materials Science, Department Of Science And Engineering Of Oxide Materials And Nanomaterials, 1 – 7, Polizu Street, 011061, Po – Box 12-134, Bucharest 1, Romania*
4 *Carol Davila University of Medicine and Pharmacy, 8 Eroii Sanitari, sector 5, Bucharest, Romania*
5 *Emergency Hospital Floreasca, Bucharest 5, Calea Floresca nr 8, sector 1, Bucarest, Romania*
6 *Horiba Jobin Yvon S.A.S., 16-18, rue du Canal, 91165 Longjumeau Cedex France*
7 *Université de Bordeaux, UFR Sciences de la Terre et de la Mer, B18, avenue des Facultés, F-33405 Talence, France*
8 *Universite Bordeaux, EA 4592 Géoressources& Environnement, EGID, 1 allée F. Daguin 18, 33607 Pessac Cedex France*

The silver doped hydroxyapatite thin films were made by thermal evaporation technique from $Ca_{10-x}Ag_x(PO_4)_6(OH)_2$powders. Commercially pure Si disks were used as the substrates. For evaporation in medium vacuum ($P \sim 8 \times 10^{-6}$torr), it was used a wolfram boat, and the intensity of the maximum current through boat was I max ~ 40 A for t~5 sec. The silver doped hydroxyapatite thin films were systematically characterized by various techniques such as X-ray Photoelectron Spectroscopy (XPS), Glow Discharge Optical Emission Spectroscopy (GDOES), Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Attachment (EDAX) and FT-IRspectroscopy. These techniques have allowed the structural elucidation of silver doped hydroxyapatite thin films. The FT-IR spectra showed clearly the presenceofthevariousvibrationalmodes correspondingto phosphates and hydroxylgroups. Glow discharge optical emission spectroscopy (GDOES) provides elemental information and was used to acquire elemental distribution from coating to substrate through depth profiling. The GDOES spectra reveal the presence of a material composed mainly of phosphate, calcium, oxygen, hydrogen and silver.Analyzing the chemical composition of the coatings with GDOES measurements revealed a homogenous distribution of the elements throughout the film.

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IRON DEFICIENT SOIL: EFFECTING CHICK PEA

E. E. Hakki[1,*], M. Hamurcu[1], A. Pandey[1], M. K. Khan[1], S. Gezgin[1]

[1]*Selcuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Konya, 42079 Türkiye*
[*]*Corresponding author's e-mail: eehakki@selcuk.edu.tr*

Scarce contents of iron in soil have a depressing impact on the progress of crops, which, in turn, influences human health. Turkey is one of the major producers of Chickpea since last several years. Therefore, to achieve its highest genetic yield it is important to restore iron deficiencies. A controlled greenhouse experiment has been performed at Selcuk University as an initiative where Chickpea plants were grown within the iron deficient soil(with 0.92 ppm Fe only). Replicates of 18 genotypes(collected from different places) were treated with 3 different doses(0, 6 and 60 ppm) of iron. Iron was applied in the form of Fe EDDHA 6%. ICP analysis of these genotypes has shown different accumulation at various doses. This research work was done to understand the effect of various iron doses on chickpea. The study can be further correlated with the molecular information for the genetic improvement of various chickpea varieties under iron deficient conditions.

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PUCCINELLIA DISTANS: IS A ROLE MODEL FOR BORON ADAPTATION?

E. E. Hakki1,*, M. Hamurcu1, T. Demiral2, Z. Z. Avsaroglu1, A. Pandey1, M. K. Khan1, S. Gezgin1

[1]*Selcuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Konya, 42079 Türkiye;*
[2]*Harran University, Faculty of Science and Literature, Sanliurfa, Türkiye*
[*]*Corresponding author's e-mail: eehakki@selcuk.edu.tr*

Among all the profitably available grasses, *Puccinellia distans* (Jacq.) Parl. is one of the most salt tolerant grass and can be grown in highly saline soils without momentous failure in production. As *P. distans* is renowned for its boron (B) tolerance feature, mainly B tolerant genotypes are those that are proficient to retain little B concentrations in their tissues. Previous research have exposed that plant tolerance to B toxicity fluctuates between species and among the cultivars of the same species. Thus, in our study, 32 *P. distans* samples were collected from 3 different locations of Central Anatolia, Turkey. The first group (12 samples) were obtained from the coasts of the artificial B mining ponds at the Eskisehir Kirka mining area. The second group (6 samples) of plants were collected from the edges of the Keceli canal of Konya which was highly polluted with the industrial and domestic wastes of Konya region. The last group (14 samples) were assembled from the non-agricultural salty arid region of Konya. In molecular analyses, 10 Inter-simple sequence repeat (ISSR) primers were utilized. In total, 114 polymorphic fragments were scored. Primer M16 had given maximum 23 bands, while minimum number of polymorphic bands was 5, obtained by M7 primer. From the results of dendogram and Principal Coordinates Analysis (PCoA), a similarity was found between the samples according to the place of collection. Those which were collected from heavy metal pollution site have been observed together in the lower branch of the dendogram showing an adaptation towards the B accumulation. These responses were later utilized to understand the physiological mechanism of tolerance of the grass plant, *P. distans*, to extremely high levels of B in growth environment with the aim of using the information from this model plant on the other economically important crops, like wheat.

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BORON TOLERANCE OF MAIZE IS CLOSELY RELATED TO ENHANCED ANTIOXIDANT SYSTEM

T. Demiral1,*, M. Hamurcu2, E. E. Hakki2, Z. Z. Avsaroglu1,2, Nihat Dilsiz1, S. Gezgin2

1 Harran University, Faculty of Science and Arts, 63300 Sanliurfa, Türkiye;
2 Selcuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Konya, 42079 Türkiye
**Correspondent author: tijen.demiral@harran.edu.tr*

A wide range of genetic variation in response to high concentrations of B occurs in a number of crops including maize. Therefore, enlightening tolerance mechanism of tolerant maize genotypes makes big sense to enable efficient strategies for breeding of B tolerant varieties to be adopted. Physiological mechanisms underlying boron tolerance in PR-3394, a hybrid variety of maize (Zea mays L.) tolerant to boron toxicity but sensitive to boron deficiency, were studied. Maize plants were grown in a growth chamber under controlled conditions. Plants grown until 3-leaf stage were exposed to 0, 2.5, 25, 50 and 100 mg L-1 boron treatments and were harvested after 0, 5 and 10 days. The effect of boron treatments on growth parameters (shoot and root), boron accumulation, proline content, malondialdehyde (MDA) content, and activities of antioxidant enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POX) and glutathione reductase (GR) were investigated. Shoot and root fresh weight and root dry weight of PR-3394 were gradually decreased by increasing concentrations of B after 10 days. 0 mg L^{-1} B treatment enhanced dry weight of shoot more than other B dosages on day 10. Shoot length of PR-3394 showed no significant change after 5-day B treatments. However, root and shoot lengths of plants decreased with increasing concentrations of B on day 10. Maize leaves accumulated more B than in roots. B treatments gradually enhanced proline accumulation in leaves. MDA content in leaves of PR-3394 was increased by 2.5 and 25 mg kg-1 B on day 5 and by 25 mg kg-1 B on day 10 whereas 50 and 100 mg kg-1 B dosages decreased MDA content on both sampling days. SOD and POX activities in leaves of PR-3394 increased after 100 mg kg^{-1} B treatment for 5 days while decreased after 25, 50 and 100 mg kg^{-1} B treatments for 10 days. CAT and GR activities were enhanced by 25, 50 and 100 mg kg-1 B treatments on both harvest days. APX activity was enhanced by 25, 50 and 100 mg kg-1 B treatments on both harvest days. These results suggested that 2.5 and 25 mg kg-1 B dosages caused oxidative stress and defense mechanism of a maize hybrid, PR-3394, against boron toxicity appeared to be related with enhanced activities of CAT, GR and APX thus decreased.

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GROWTH RESPONSES OF A MAIZE GENOTYPE, TOLERANT TO BORON DEFICIENCY BUT SENSITIVE TO BORON TOXICITY, AGAINST VARIOUS B DOSAGES

M. Hamurcu[1,*], T. Demiral[2], E. E. Hakki[1], Z. Z. Avsaroglu[1,2], H. Can[1]S. Gezgin[1]

[1]*Selcuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Konya, 42079 Türkiye;*
[2]*Harran University, Faculty of Science and Arts, 63300 Sanliurfa, Türkiye*
[*]*Correspondent author: mhamurcu@selcuk.edu.tr*

Boron is an essential nutrient for optimal growth of higher plants and its availability in environment is an important determinant of agricultural production and yield. Boron deficiency and toxicity symptoms of a maize hybrid DK647, tolerant to boron deficiency but sensitive to boron toxicity, were studied. DK647 seedlings were grown until they are 3-leaf stage in a growth chamber under controlled conditions. Plants were treated with Hoagland solution containing 0, 2.5, 25, 50 and 100 mg L^{-1} boron for 5 or 10 days and the effect of various concentrations of B on growth parameters (shoot and root), leaf relative water content (RWC), boron accumulation level and proline content were investigated. Shoot and root fresh weights were decreased especially by 50 and 100 mg L^{-1} B treatments for 5 and 10 days. Dry weight of shoot and root was decreased by 100 mg L^{-1} B treatments on both sampling days. Lengths of shoot and root were decreased by 50 and 100 mg L^{-1} B treatments on both harvest days and the longest values were observed in 0 mg L^{-1} B treatment group. Highest value of leaf RWC was observed with 0 mg L^{-1} B treatment and no remarkable decrease was observed in leaf RWC after 2.5, 25, 50 and 100 mg L^{-1} B treatments. Boron accumulation level was increased in parallel with the enhancing concentrations of B treatments. B accumulation in shoots reached 5695.7 mg kg^{-1} while it reached 1369.6 mg kg^{-1} in roots after treating DK647 plants with 100 mg L^{-1} B treatment for 10 days. Increasing concentrations of B treatment promoted proline accumulation in shoots and the highest level of proline accumulation was observed in 50 mg L^{-1} B treatment group. Accumulation level of proline seemed not enough to provide osmotic adjustment. These results suggest that deficient and toxic concentrations of boron remarkably affects growth and physiological responses of maize hybrid DK647.

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MONITORING OF PARTICULATE MATTER IN AMBIENT AIR AT TWO DIFFERENT LOCATIONS IN PORTO METROPOLITAN AREA (PORTUGAL) – FOCUS ON TRACE ELEMENT DIFFERENCES AND ANTHROPOGENIC SOURCES OF CONTAMINATION

Cristina Couto 1,3, Carlos Soares 1, Rui Ferreira 1, Edgar Pinto 2, Agostinho Almeida 1

1 REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal.
2 REQUIMTE, Department of Chemical Sciences, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal.
3 CICS, Centro de Investigação em Ciências da Saúde, Instituto Superior de Ciências da Saúde-Norte, 4585-116 Gandra, Paredes, Portugal. cristina.couto@iscsn.cespu.pt

The effects of ambient particulate matter (PM) on health are well recognized. Several epidemiological studies have demonstrated a clear relationship between increased mortality, hospital admissions, and respiratory illness and air PM levels. Unfortunately most of these studies rely solely on a gravimetric analysis, even though it is increasingly recognized the importance of also considering PM chemical composition. This work is part of a more comprehensive Project intended to assess environmental risk factors for cardiovascular diseases at Porto Metropolitan Area (PMA). Using sampling recommended procedures PM10 and PM2.5 was collected at two different sampling sites (SS) in PMA (SS1 – Center of Porto city; SS2 – A peripheral area: Gandra-Paredes, ca. 20 km NE from Porto). A wide range of metals and metalloids were determined in sampling filters extracts (obtained by sonication for 3 h with 20 mL of 4% nitric acid at 69 ºC) by ICP- MS. For the most abundant trace elements, the PM content approximately followed the same order on both SS (Al > Zn > Cu > Pb > Mn on PM10; Zn > Al > Cu > Pb on PM2.5), but notable differences were found for the other. Levels at SS1 were 3-5 times higher than at SS2, but even here the data obtained fully comply with the EU Air Quality Standards (Directive 2008/50/EC) regarding the current limit value for Pb and the target values for As, Cd and Ni entered into force on 31.12.2012. Cr, As, Se, Cd e Pb, at SS1 (and also Ni, Cu and Zn at SS2), were mostly (>70%) associated with PM2.5, which indicates specific sources for the air contamination with these metals. PM2.5 are mainly produced by combustion processes while PM10 particles mainly originate from soil dust resuspension, road transport and industrial processes (including bulk handling, construction, mining and quarrying).

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THE MAINTENANCE OF TRACE ELEMENTS IN THE SOILS AND PLANTS GROWING IN THE TERRITORY OF THE FORMER SEMIPALATINSK PROVING NUCLEAR POLYGON (KAZAKHSTAN)

K.O. Sharipov, S.M. Pleshkova, A.A. Kirgizbayeva, E.O. Muchamadiea

skamalidin@mail.ru

Kazakh national medical university. Named after C.D. Asfendiyarov
Almaty, Kazakhstan

We investigated the trace element composition of soil and plant samples taken at the former Semipalatinsk nuclear test site (SNTS, Kazakhstan). Trace element composition was determined by emission spectrometry quantitative method for DFS-8 spectrograph.

The soil of Abralinsk area was containing copper, zinc and manganese less compared with soils in other regions. In the mountain range Degelen copper contained 10 times higher than the soils of other regions and is 8.5 times higher than the maximum permissible concentration (MPC). The soil of Atomic Lake and Experienced Field characterized by a high content of manganese and exceed the same indicators in the soils of other regions in the 2.5-5.5 times. The concentration of zinc in soils of Atomic lake and Experimental Field exceeded than the soils of other regions - in 1,5 times. The copper content in the soils of the Atomic Lake and Experienced Field exceeds the copper content in the soils of other regions in the 2 times. The observed excess of lead in soil Experimental Fields and Degelen, which can be explained by effects of nuclear explosions. Plants of Abralinsk area characterized by a high content of chromium (23 times), scandium (7 times), copper (3 times), aluminum (2 times), vanadium (2 times). Plants with mountain massif Degelen are rich scandium, chromium and molybdenum. The content of chromium outweighs the world values (23 times), molybdenum (8 times) and scandium (7 times). In plants which taken from Experienced Field, showed an increase of chromium (23 times), scandium (5 times), lead (4-fold) and molybdenum (2 times). Samples taken from the epicenter of explosions Experimental fields and Degel, characterized by a high content of lead in plants that exceed the lead content in comparison with the world data in 2 times, this is due to the fact that lead is the final degradation product of U-238. The results of our investigations showed that the content of trace elements in soils and plants, taken from Semipalatinsk proving ground are significantly different from the world data (Clarke). The plants and soil Abralinsk area are rich in copper, aluminum, and iron. In plants and soils Degel are most contained molybdenum, manganese, zinc, and nickel. Experimental Field and Atomic Lake characterized content in plants and soils of lead, which is probably related to the type of bombs tested at the landfill site.

16. Animals

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BLOOD MINERAL STATUS IN LAMBS EXPERIMENTALLY INFECTED WITH HAEMONCHUS CONTORTUS (NEMATODA)

Veselin Nanev, Ivelin Vladov, Milena Anissimova, Margarita Gabrashanska

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, e-mail:veselinnanev@gmail.com

The blood sucking parasite Haemonchus contortus causes huge economic losses due to lowered meat and wool production, hides. It is found in the abomasum of the sheep and goats and is responsible for acute outbreaks with mortalities, particularly in young animals.

The aim was to establish serum mineral status in lambs experimentally infected with Haemonchus contortus. The levels of Ca, Na, Fe, Cu, Zn and Se were determined in blood of lambs infected and non-infected with H. contortus.

The lambs of 3-4 months of age were studied. They were allocated into 2 groups on body weight basis – control and infected with H. contortus. The second group was infected two times every two days with 1800 H. contortus larvae (L3) per a lamb. The Baermann technique was used to extract the L3 larval stages of H. contortus intestinal nematodes and counted under a dissecting microscope to determine the larval counts. Blood was obtained on day 58 post infection. The levels of elements were determined by an atomic absorption spectrophotometry. The statistical analysis was carried out on the Prism 4.0 program.

The mean phosphorous level of infected lambs failed to show any consistent pattern of changes throughout the observation period. On the other hand, serum calcium was significantly decreased when compared to that of control animals. Mean serum iron of infected lambs was significantly reduced when compared with the non-infected controls. Analysis of serum electrolytes indicated significant decline of serum sodium. The levels of Zn, Cu and Se were reduced in the serum of infected lambs compared to non-infected ones.

The results showed the roles of the biogenic elements in the interaction between gastrointestinal nematodes and their mammalian hosts. They confirmed that gastrointestinal nematode infections lead to macro- and micro-nutrient imbalance – deficiencies which in turn increase susceptibility to infection, primarily though impairment of immune and inflammatory responses.

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TEMPORAL VARIATIONS IN TRACE ELEMENTS PROFILE DURING FOLLICULAR ATRESIA IN CAPRINE OVARY

Jitender Kumar Bhardwaj

Department of Zoology, Kurukshetra University, Kurukshetra-136119 Haryana, India
jkbkuk@gmail.com

The reproductive performance of any mammalian species can be enhanced by enriching nutrition, environmental factors like photoperiod, temperature, humidity etc., through selective breeding, endocrine manipulations and better management practices (Bhardwaj and Sharma, 2011). The development and growth of the ovarian follicles is finely regulated by a complex interaction of extracellular and intracellular factors, any deviation thereof leads to follicular atresia. Minerals are essential for growth and reproduction as they are involved in a number of physiological and biochemical processes within the body. They perform many important functions for the maintenance of animal growth and reproduction by acting as electrolytes and as catalysts in both enzyme and hormone systems. Trace elements have also traditionally been known to play an important role in cellular homeostasis which entails the tight regulation of cell death. The role of trace elements as pro-apoptotic or antiapoptotic factors is yet to be established. Therefore, in the present study quantitative analysis of follicular fluid and granulosa cells from small, medium and large antral follicles of goat (Capra hircus) was conducted to study the variations in Zinc (Zn), Copper (Cu), Manganese (Mn) and Iron (Fe) content to provide an index for designating the status of the follicle and thus is helpful in reducing losses during in vitro fertilization and Embryo transfer technology. The zinc content was estimated as 0.993 ± 0.001, 0.935 ± 0.002, and 1.321 ± 0.001 µg/ml in the follicular fluid of small, medium and large antral atretic follicles and was 1.213 ± 0.002, 1.118 ± 0.002, 1.394 ± 0.002 µg/ml in the follicular fluid of small, medium and large normal antral follicles. In the granulosa cells of all the three categories of antral atretic follicles the zinc content was 0.867 ± 0.002, 0.801 ± 0.001, 1.073 ± 0.002 µg/100mg respectively and 1.045 ± 0.001, 1.086 ± 0.002, 1.092 ± 0.001 µg/100mg in the granulosa cells of normal antral follicles respectively. Thus, the zinc content was lower in the follicular fluid and granulsoa cells of all the three categories of antral atretic follicles as compared to those of normal follicles.

The quantity of copper was 0.113 ± 0.001, 0.163 ± 0.001, 0.224 ± 0.001 µg/ml in the follicular fluid and 0.094 ± 0.001, 0.114 ± 0.001, 0.182 ± 0.001 µg/100mg in the granulosa cells of small, medium and large antral atretic follicles respectively. The copper content was 0.107 ± 0.001, 0.118 ± 0.001, 0.172 ± 0.001 µg/ml in the follicular fluid and 0.081 ± 0.001, 0.093 ± 0.001, 0.162 ± 0.001 µg/100 mg in the granulosa cells of small, medium and large normal follicles respectively. The quantity of copper was higher in the follicular fluid and granulosa cells of atretic antral follicles as compared to the normal follicles.

The quantity of Manganese was 0.865 ± 0.001, 0.574 ± 0.002, 0.380 ± 0.001 µg/ml in the follicular fluid and 0.793 ± 0.001, 0.651 ± 0.083, 0.194 ± 0.001 µg/100mg in the granulosa cells of small, medium and large antral atretic follicles respectively. The manganese content was 0.796 ± 0.001, 0.135 ± 0.001, 0.134 ± 0.001 µg/ml in the follicular fluid and 0.773 ± 0.008, 0.533 ± 0.001, 0.173 ± 0.001 µg/100mg in the granulosa cells of small, medium and large normal antral follicles respectively. The quantity of manganese was higher in the follicular fluid and granulsoa cells of atretic antral follicles as compared to the normal follicles.

The Iron content was estimated as 1.336 ± 0.002, 1.452 ± 0.001, 2.340 ± 0.070 µg/ml in the follicular fluid and 1.952 ± 0.001, 2.076 ± 0.002, 2.083 ± 0.002 µg/100mg in the granulosa cells of small, medium and large antral atretic follicles respectively. The Iron content was 1.311 ± 0.001, 1.256 ± 0.002, 1.162 ± 0.001 µg/ml in the follicular fluid and 1.563 ± 0.001, 1.502 ± 0.002, 1.052 ± 0.001 µg/100mg in the granulosa cells of small, medium and large normal antral follicles respectively. Thus, the quantity of Iron was higher in the follicular fluid and granulosa cells of atretic antral follicles as compared to the normal follicles. From the findings of the present study, it is concluded that zinc specific enzyme activity declines during atresia which is concomitant with the diminishing levels of estrogen. The rising levels of copper during atresia are possibly due to some alterations in gonadotropins/hormonal balance inducing catabolic changes. The rising level of iron content during atresia is an index of increased vascularity for clearing the debris. The variations in manganese content are indicative of its variations in enzyme activities. Therefore, it is to say that the trace element content can be used as an index of metabolic activities of the follicle. The present study provides the basic data on trace elements that can be safely used as atretic marker and will find use in in vitro studies for fertility improvement plan. Thus, help in elevating the number of ovulations and screening of follicles to enhance the success rate in vivo and in vitro fertilization and embryo transfer technology.

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INVESTIGATION OF TRACE ELEMENTS IN THE SALIVARY GLAND SECRET AND IN THE TISSUES OF THE MEDICAL LEECH (HIRUDO VERBANA CARENA)

L.V. Chernaya, L.A. Kovalchuk

Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences
8 Marta Str., 202, Yekaterinburg, 620144 Russia.
kovalchuk@ipae.uran.ru

The present medicine widely uses the combination of traditional and untraditional methods in the treatment and prevention of diseases. Undoubtedly, that the medical leech is an officially recognized medical means. This paper reports on the content of trace elements in tissues and salivary gland secret of the leeches reared at the "Medical Leech International Centre" (Moscow region, Udelnaya settlement), the starvation period being 5 months. 100 adults were used in the research. We compared the levels of biologically active compounds in the salivary glands secret and in the tissues. It was established that the total content of trace elements in the tissues 6.4 times exceeded that in the salivary gland secret. The formation spectrum of the bioelements in the salivary gland secret was aimed at a high percentage of essential elements (Ca, Mg, Zn), Ca accounting for 87.5%. The salivary gland secret had much less Fe (0.76%) than tissues (31.7% in homogenates). The content of leech exotoxicant (Co, Cd, Ni, Pb) in the salivary gland secret was a hundredth part of a percent from the total thanks to a depositing function of tissues. The content of toxic metals (Ni, Cd, Pb) in the salivary gland secret of the medicinal leeches was two orders lower than in the tissues. The results of the research may be used for more effective application of bioelements in the tissues and in the salivary glands secret of the medical leech in the production of the pharmaceutical, food biological, cosmetics, biological additives.

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SELENIUM CONTENT OF ALGERIAN CAMEL MEAT

Sahraoui N1., Dotreppe O.2, Brahim Errahmani M. 1, Boudjenah S.3, Babelhadj A.3, Guetarni D1 ., Hornick JL. 2

1 : Université de Blida –Algérie
2 : Service de nutrition-Université de Liège-Belgique
3 : Université de Ouargla- Algérie
Email address: nasahraoui@gmail.com

The dromedary is able to produce meat and milk in arid conditions and its production is appreciated by the autochthon population.

This study focuses on the determination of selenium content in meat samples obtained at the slaughterhouse of Ouargla (South East Algeria) from 41 males and 20 females camels from Tergui, Sahraoui or Naili breeds, aged between 8 months and 13 years old.

The average content of selenium was 21,6 µg/100g of wet tissue, what is higher than values currently reported in ruminants .The level of Se was not influenced by age and sex but Tergui breed showed higher values than the two other breeds. No interaction effect was found between the parameters. This breed effect could result from difference in feed composition, but the hypothesis should be confirmed by further research.

In conclusion, the meat from camelids in Algeria is a good source of selenium, confirming its nutritional interest for human.

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TRACE METALS (CU, FE, MN, ZN AND CD) LEVELS IN A TRIPLEX: HEALTHY AND NUTRITION IMPLCATIONS IN RUMINANTS

Arab H.1*, Haddi M-L2, Mehennahoui S1

1 *Environment, Health and Animal Production Laboratory.Veterinary and Agronomy Institute. Veterinary Department .University Hadj Lakhdar. Batna. Algeria.*
lrespa@yahoo.fr
2 *Department of Biochemistry Microbiology. Faculty of Natural Sciences and life. University Mentouri Constantine. Algeria. haddil@yahoo.com*
arab_hadda@yahoo.fr

Objectives:

Determine the levels of essential minerals (Cu, Zn, Mn and Fe) and non-essential (Cd) in a halophyte shrub *Atriplex halimus* ground in arid zone of Algeria in order to assess deficiencies or excesses and toxic effects on rumen flora.

Materials and Methods:

The forage was collected in the arid zone of Biskra situated in South of Algeria, sampled for ten campaigns and covers an annual cycle. The stems and leaves are manually separated and divided each into five parts to assess the distribution of mineral elements. Cu, Fe, Mn and Zn are measured with flame Atomic Absorption Spectrophotometry, Cd with furnace.

Results and discussion:

Cu, Zn, Mn and Fe mean levels for stems were respectively:12, 53, 8 and 436mg/kg of MS and for leaves were respectively : 14, 69, 27 and 741mg/kg of MS. Cd levels ranged from 50 to 489 µg/kg DM and from 44 to 331 µg /kg of MS respectively in stems and leaves.

The differences observed between the stems and leaves are not statistically significant for Cu and Cd (T-test Student $p > 0.05$), and significant for Zn ($p < 0.01$), Fe and Mn (($p<0.001$). Moreover, the distribution of minerals in the different fractions of stems and leaves is variable and does not present a clear trend. Level of Cu presents a health hazard to ruminants especially sheep. However, several factors may penalize the absorption of this element (such excess Fe, Ca and Zn) which makes it less bioavailable. Cd contents ranged from close to slightly higher to the background level. The higher value of Cd in forage may affect the activity of rumen flora and therefore health risk.

Conclusion:

Our results showed that the mean levels of trace metals in *Atriplex halimus* are; high for iron, close to the value recommended by INRA for Cu and Zn, deficient for Mn and close to the background level for the Cd.

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STUDY OF THE MINERAL COMPOSITION OF THE SHELL EMBRYONIC EGG

B. Beddiaf1 ; M. Melizi2

1 *Institut Of Veterinary Sciences And Agricultural Sciences, Environment, Health and Animal Production Laboratory, Hadj Lakhdar Batna University, Algeria*
bani_b90@hotmail.com
2 *Institut Of Veterinary Sciences And Agricultural Sciences, Environment, Health and Animal Production Laboratory, Hadj Lakhdar Batna University, Algeria*
mohamed.melizi@yahoo.fr

Considering calcium is not the only mineral exhausted by the embryo during the embryonic development, we propose to evaluate the rate and the behavior of minerals that constitutes the shell of embryonic egg.

For that; a study of the mineral composition of the shell of embryonic egg is carried out according to the embryonic development stages, then compared with that of egg for consumption.

Incineration and acid-attack procedure were performed on the embryonic-egg shells at the stages: 1 , 7, 14, and 21 days of incubation, to determine calcium, magnesium, manganese, zinc and copper content by atomic absorption spectrophotometry, whereas the phosphorus content was determined by visible spectrophotometry.

Our results are largely lower than those reported in the bibliography with regard to the average content of the embryonic-egg shells of manganese magnesium and calcium. While, the rate of copper and zinc increased from 1st to the 21st day of incubation; phosphorus presented a behavior independent of other minerals with a stable rate throughout the incubation period.

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EFFECTS OF DIFFERENT LEVELS OF DIETARY MN AND ZN ON THE DISTRIBUTION OF THESE ELEMENTS IN THE BLOOD, TIBIA, LIVER, EXCRETA AND EGGS

RD Criste1, AE Untea1, GM Cornescu2,1, M Olteanu1

1 National Research and Development Institute for Biology and Animal Nutrition (IBNA- Balotesti), 077015, Calea Bucureşti, 1, jud. Ilfov, Balotesti, Romania, tel. (+40 21) 351. 20.82; Fax (+40 21) 351. 20.80; e-mail: criste.rodica@ibna.ro; cristerodica@yahoo.com
2 University of Agronomic Sciences and Veterinary Medicine in Bucharest (UASVMB); Bvd. Marasti, no. 59, Bucharest, Cod: 011464

An experiment was conducted for 6 weeks on 160 Lohmann Brown layers, aged 22 weeks, housed in cages (3 layers/cage), with a light regimen of 16 h/day. The layers were assigned to 4 groups (C, E1, E2 and E3), which received the same basal diet with 2724 kcal/kg metabolisable energy; 17.96% crude protein; 3.79% Ca and 0.68% P. The diets differed by the dietary Mn and Zn levels provided by mineral premixes. There is literature data which shows that supplementing layer diets with Mn and Zn, individually, or together, favours better eggshell quality and even enriches the egg in these elements.

The commercial premix for diet C included 7190 (mk/kg) Mn and 6000 (mg/kg) Zn. Compared to the premix for C group, the premixes of the experimental groups had higher concentrations (mg/kg) of these trace minerals : 8500 Mn and 14500 Zn (E1); 10000 Mn and 8000 Zn (E2) 20000 Mn and 16000 Zn (E3). Throughout the experiment there were recorded on a daily basis the feed intake of the groups and the egg production. Every two weeks we collected randomly 18 eggs/group. After the physical parameters of quality were determined for collected eggs, there were formed 6 samples/group of egg yolk, egg white and egg shell. In the end of the experiment there were also collected blood samples and 6 hens from each group were thereafter slaughtered and bone and liver samples were collected. The feed samples and all collected samples were assayed for Mn and Zn. The samples of feed, egg shell and bone were also assayed for Ca and P. The experimental results showed a good correlation between the dietary Mn level and the serum (0.780) and yolk Mn (0,7694) levels. The dietary Zn level had a good correlation with the yolk Zn level (0.849) and rather well with the eggshell Zn level (0.623). The yolk Mn concentration (2,89 ± 0,32 mg/kg dry matter) and yolk Zn concentration (81,19 ± 1,07 mg/kg dry matter) were significantly (P≤ 0.05) higher than in C group (2,23 ± 0,35 mg/kg Mn; 78,51 ± 1,62 mg/kg Zn).

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MICRO ELEMENTS LEVELS IN THE MILK OF HORSES AND DONKEYS IN CROATIA

Nina Bilandžić, Marija Sedak, Maja Dokić, Ivana Varenina, Božica Solomun Kolanović, Durdica Božić

Laboratory for Residue Control, Department for Veterinary Public Health, Croatian Veterinary Institute, 10000 Zagreb, Croatia
e-mail: bilandzic@veinst.hr

The concentrations of micro elements Al, Co, Cr, Li, Mo, Ni, Sb and Sr in the milk of Croatian Coldblood horse and Littoral-Dinaric donkey were determined by inductively coupled plasma-optical emission spectrometry. The total mean element contents in horse milk were (μg/kg): Al < 10, Co < 10, Cr 27.3, Li 4.98, Mo 12.1, Ni < 10, Sb 67.2 and Sr 495.5. Variation of Li, Sb and Sr concentrations were found throughout the lactation stages (days: 10, 40, 60, 120, 180). In donkey milk, the mean element contents were (μg/kg): Al < 10, Co < 10, Cr 26.1, Li 8.39, Mo 8.98, Ni < 10, Sb 24.1 and Sr 560.4. Correlations were found among elements in horse and donkey milk. Significant differences in concentrations of Li and Sb between two species were determined. Other elements concentrations were similar in both species. Very limited data are available on the micro element content of Al, Cr, Co, Li, Mo, Ni, Sb and Sr in horse and donkey milk in general. To our knowledge there is a first report on these micro elements content in milk of these two species in Croatia.

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EFFECT OF CRPIC SUPPLEMENTS ON CARCASS TRAITS OF GROWING-FINISHING PIGS

Arabela Elena Untea1, Rodica Diana Criste1, Tatiana Dumitra Panaite1, Mariana Ropota1, Camelia Papuc2, Predescu Nicoleta Corina2

1 *National Research and Development Institute for Biology and Animal Nutrition (IBNA Baloteşti), 077015, Calea Bucureşti, 1, jud. Ilfov, Balotesti, Romania,*
2 *Faculty of Veterinary Medicine, 105 Splaiul Independentei, 050097, Bucharest, Romania.*

A 4-week study conducted on growing-finishing pigs evaluated the effects of the chromium picolinate (CrPic), on the quality of the pig breast and muscle meat. The experiment was conducted under farm conditions, on 18 castrated, Landrace × Large White pigs, with an average initial body weight of 73.55 kg/pig. The pigs were assigned to three groups (C, E1 and E2) and received the same basal diet with corn (27%) and soybean meal (13%). Compared to C, the experimental diets were supplemented with 200 ppb CrPic (E1) and 400 ppb CrPic (E2). At the end of the experiment samples of blood were collected and thereafter all pigs were slaughtered and breast and muscle meat samples were collected. The samples were assayed for the crude fat, crude protein, cholesterol, fatty acids profile, amino acids profile and thiobarbituric acid reactive substances (TBARS) in order to evaluate the carcass traits. The haematological and biochemical parameters determined in the end of the experiment showed a proper health state of the pigs. The post-slaughter measurements have shown that the fat layer of the pig carcass was smaller in E2 (14.33 mm) than in E1 (16.50 mm) and C (17.17 mm). The breast meat samples revealed a significantly (P≤0.05) lower fat proportion (48.18%), and a significantly (P≤0.05) higher protein level (40.40%) in group E1 compared to C group (59.13% fat; 28.92% protein) and E2 group (60.36% fat; 36.37% protein). TBARS determinations on the samples of breast meat have shown that the supplemental 200 ppm Cr decreased TBARS value from 0.634 (group C) to 0.518 mg MDA/kg, while the supplemental 400 ppm Cr didn't inhibit lipid, the value of TBARS being 0.633 mg MDA/kg. The fatty acids profile didn't differ significantly between the three groups, for any type of sample.

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CORELLATIONS CONCERNING THE CONTENT OF CU AND MN FROM WEANED PIGLETS DIETS, SUPPLEMENTED WITH PLANTS HARVESTED FROM THE WILD FLORA AND THEIR RETRIEVAL IN ANIMAL ORGANISM

Untea Arabela Elena, Criste Rodica Diana, Panaite Tatiana Dumitra,Voicu Ilie, Mircea Eugenia

National Research and Development Institute for Biology and Animal Nutrition (IBNA Baloteşti), 077015, Calea Bucureşti, 1, jud. Ilfov, Balotesti, Romania,

A 4-week study conducted on 15 weaned piglets evaluated the effects of the dietary oregano (Origanum vulgare L.) or mixture of plants (oregano and bilberry (Vaccinum Myrtillus L.)), on Cu and Mn retrieval in animal organism, while eliminating their inclusion in the diet as inorganic salts. The harvesting area for oregano was Romania, Vâlcea County – Păuşeşti – Otăsău; and the vegetal material of bilberry, was harvested from Romania, Gorj County, Parâng Massif.

The piglets were assigned to 3 groups (C, E1, E2), housed in individual metabolic cages and fed on corn-soybean meal-based diets. The control group diet (C), contained vitamin – mineral premix (1%) which included 25 mg/kg Cu and 31 mg/kg Mn from inorganic salts (sulphates). The experimental group 1 diet (E1) included 3% oregano and a vitamin –mineral premix (1%) with a similar structure as the premix used for the control group, but without Mn and Cu salts. The experimental group 2 diet (E2) included 3% oregano and 1% bilberry, and a vitamin –mineral premix (1%) with a similar structure as the premix used for the control group but without Mn and Cu salts. Due to the total reduction of Mn and Cu from these inorganic sources, their content from the experimental diets were: 9.46 mg/kg Cu and 17.08 mg/kg Mn for E1 group; 9.39 mg/kg Cu and 19.22 mg/kg Mn for E2 group compared to 34.21 mg/kg Cu and 45.69 mg/kg Mn for C group.

The results showed that there are no significant differences ($p \leq 0.05$) between experimental groups concerning the average daily gains (C: 0,567 kg/day ± 0,35; E1: 0,540 kg/day ± 0,28; E2: 0,547 kg/day ± 0,26) and the average daily intakes (C: 1,30 kg/day ± 0,27; E1: 1,25 kg/day ± 0,25; E2: 1,32 kg/day ± 0,18). The balance was performed for 5 days every week. The Cu and Mn were determined by FAAS in the samples (weekly samples/piglet) of ingesta, faeces, urine, serum and organs (liver, spleen, kidney, heart, brain, lungs).

The amounts of Cu and Mn deposited in the organs during the experimental period were significantly higher for group C compared to experimental groups (0.23 mg Cu/animal/day for C group compared to 0.15 mg Cu/ animal /day for E1 and 0.12 mg Cu/ animal /day for E2, respectively 0.12 mg Mn/ animal /day for C group compared to 0.09 mg Mn/ animal /day for E1 and 0.09 mg Mn/ animal /day for E2). The ratio between the amount of Cu and Zn deposited and the amount of Cu and Zn retained in the body, showed an efficient

utilization of the diets supplemented with plants from the wild flora compared to a commercial diet (for Cu: 0.84% for C group and 3.21 respectively, 3.57% for experimental groups and for Mn: 0.33% for C group and 1.59 and respectively 1.47% for experimental groups)

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